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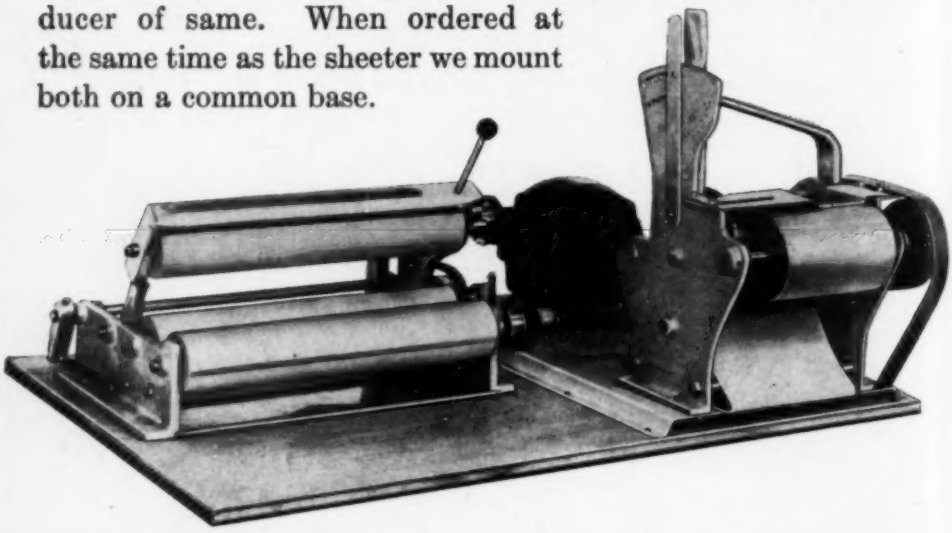

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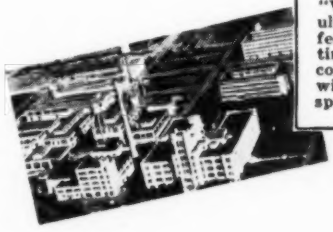

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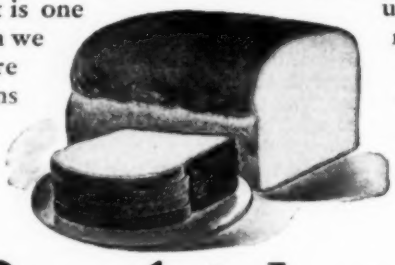

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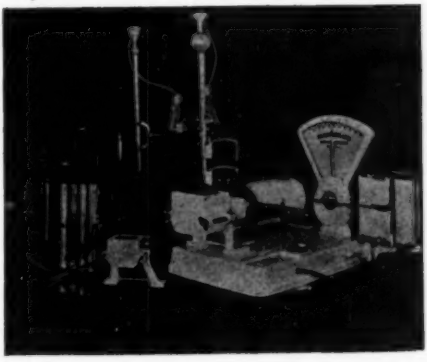

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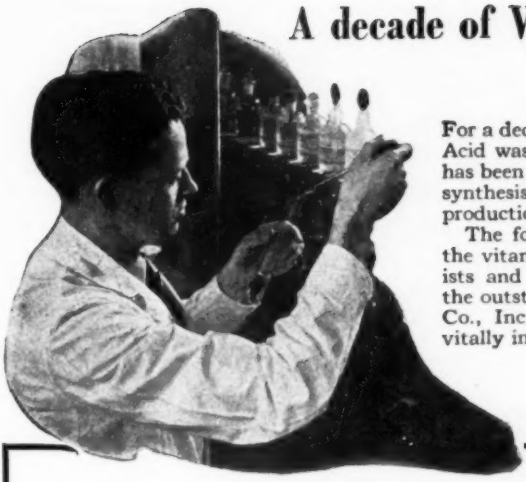

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1938
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1938
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1939
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1940
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1940
Alpha-Tocopherol Merck (Vitamin E) was made commercially available.

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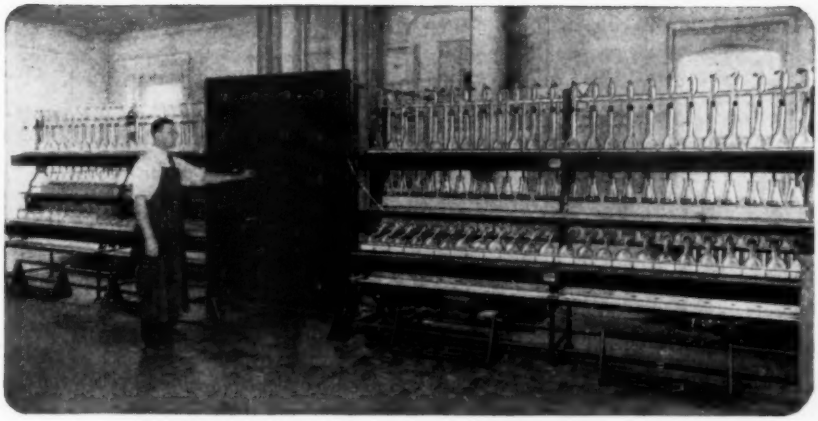

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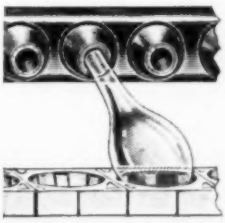

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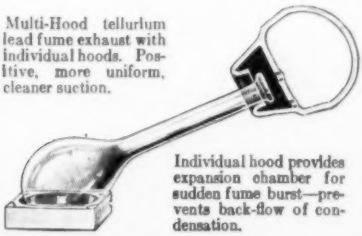

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CEREAL CHEMISTRY

VOL. XXI JULY, 1944 NO. 4

DOUGH OXIDATION AND MIXING STUDIES. VI. EFFECTS OF OXIDIZING AGENTS IN THE PRESENCE OF REDUCING MATTER

J. FREILICH AND C. N. FREY

The Fleischmann Laboratories, Standard Brands Incorporated, New York, N. Y.

(Presented at the Annual Meeting, May 1943; received for publication November 28, 1943)

The importance of reducing matter as a factor in bread quality has been well established. Sullivan and co-workers (1936) showed that glutathione is present in wheat germ. Quantitative estimations of reducing matter in different grades of flour have been made in our laboratory (Freilich, 1941) and similar results have been reported by Shen and Geddes (1942). It has also been amply demonstrated that the harmful effects of reducing substances such as glutathione may be eliminated by the use of oxidizing agents in dough.

The Farinograph and similar apparatus have been found useful by different investigators in studying the effects of reducing matter in dough. Sullivan (1936) and Ford and Maiden (1938) obtained Farinograph curves indicating a direct effect of glutathione on gluten. Ziegler (1940) observed that increasing increments of glutathione in dough decreased the swelling time, or time to maximum consistency, in the Farinograph. Swanson and Andrews (1943), with the aid of mixograph curves, found that cysteine and other substances may greatly decrease the mixing periods.

Ziegler (1940, 1940a, 1940b) approached the problem with the apparent purpose of explaining the improving effects of bromate in wheat flour doughs, in which glutathione is believed to be the most harmful factor.

In studies on the rate of oxidation of pure glutathione in water solution, he found that at ordinary temperatures bromate oxidized glutathione very slowly, while iodine oxidized it immediately (1940a). Assuming that the action in dough is similar to that in water solution, he concluded that the improving effects of bromate "can be explained by its slow rate of oxidation of glutathione."

Ziegler (1940a) studied the effects of oxidized glutathione in dough. He assumed that "through oxidation by bromate . . . a certain amount of oxidized glutathione will exist in that dough." He found that oxidized glutathione shortened the swelling time (mixing time) and improved loaf volume. He concluded that the improving effects of bromate in dough are due (*a*) to the suppression of the harmful effect of (reduced) glutathione, and (*b*) partly due to the presence of oxidized glutathione in dough.

Ziegler (1940b) also studied the oxidation of glutathione by different oxidizing agents, using Farinograph curves to illustrate some of the results. Reduced glutathione produced rapid breakdown of the dough during mixing, suggesting a specific—SH effect. Similar gluten breakdown was produced when using glutathione oxidized with iodine, and partly oxidized with bromate, in fermenting and nonfermenting doughs, showing that flour constituents do not appear to increase the rate of oxidation of glutathione by bromate, and suggesting that "the oxidized glutathione seems to be reduced again in the nonfermenting dough." Very much more persulfate than bromate was needed to produce similar oxidation of glutathione. This leads Ziegler to say, "It would seem that although bromate action in baking can be explained to a certain extent by its oxidation of GSH, the improvement by persulphate is mainly due to action on other factors."

In our work with glutathione (Freilich and Frey, 1939), we have made reference to its immediate or specific effects, observable during dough mixing. The Farinograph curves in Figure 1 show the effects of glutathione during dough mixing and on remixing the same doughs after fermentation. It is evident that glutathione produced changes in consistency, the slope of the curve, and the time required to reach maximum consistency.

The mixing time results for Figure 1 are shown in Table I.

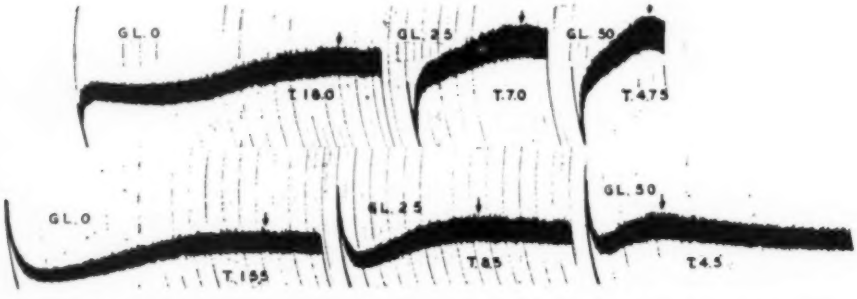

Fig. 1. Effects of varying amounts of glutathione (GL.) in dough; the upper curves show the original mixing of the doughs, and the lower curves show the remixing of the same doughs after a normal fermentation period; the point of maximum consistency (T.) is indicated by the small arrow above each curve.

TABLE I

EFFECTS OF GLUTATHIONE ON DOUGH MIXING TIME AND ON REMIXING TIME AFTER FERMENTATION

Glutathione per 300 g flour	Mixing time	Remixing time
mg	*min*	*min*
0	16.0	15.5
25	7.0	8.5
50	4.75	4.5

These effects of glutathione suggested the possibility of studying the interaction between oxidizing agents and reducing matter in dough. It seemed reasonable to expect that if reducing matter in the dough were oxidized its effects in mixing would be eliminated.

Experiments with glutathione and bromate were conducted accordingly. Table II shows the Farinograph remixing time to maximum consistency and the loaf volume for a series of sponge doughs.

TABLE II

EFFECTS OF GLUTATHIONE AND BROMATE ON LOAF VOLUME AND FARINOGRAPH REMIXING TIME OF SPONGE DOUGHS

Treatment per 300 g flour		Loaf volume	Remixing time
Glutathione	Potassium bromate		
mg	*mg*	*cc*	*min*
0	0	2080	12.75
25	0	1880	6.75
25	5	2160	5.25
25	10	2200	5.0
25	25	2150	4.75

Figure 2 shows the Farinograph remixing curves for these sponges.

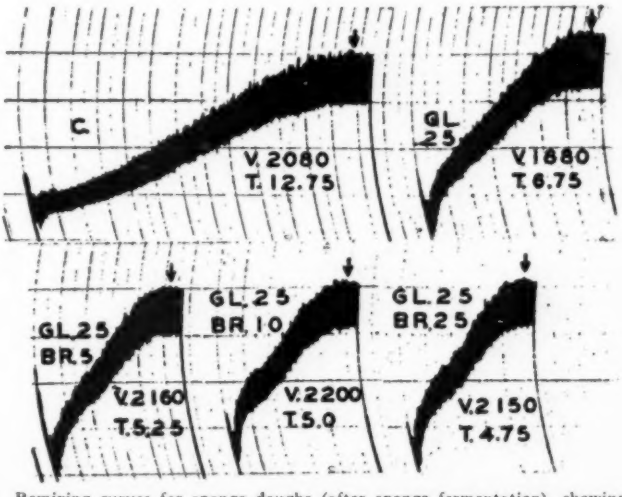

Fig. 2. Remixing curves for sponge doughs (after sponge fermentation), showing the effects of glutathione (GL.) and bromate (BR.) on remixing time (T.); the loaf volume figures (V.) from Table II are also shown; C. is the control curve.

It is evident that 5 mg of bromate was enough to overcome the decrease in loaf volume due to glutathione. It is also apparent that glutathione produced a marked decrease in mixing time, but bromate did not eliminate this mixing time effect; instead, it produced a further decrease in the mixing time, even when using five times the quantity which eliminated the loaf volume effect.

This result indicated the probability that there was no direct oxidation of reducing matter by oxidizing agents in dough, and further study was undertaken in an attempt to learn more about the relationships between the different factors involved in the oxidation-reduction systems in dough.

Effects of Bromate on Remixing Time of Sponge Doughs

In view of the previous results with bromate, experiments with bromate alone were conducted to note its effects on remixing time.

The results are shown in Table III and Figure 3.

TABLE III

REMIXING TIME OF SPONGES WITH VARYING AMOUNTS OF BROMATE

Potassium bromate per 300 g flour	Remixing time
mg	*min*
0	10.0
5	9.0
10	8.5
25	7.25

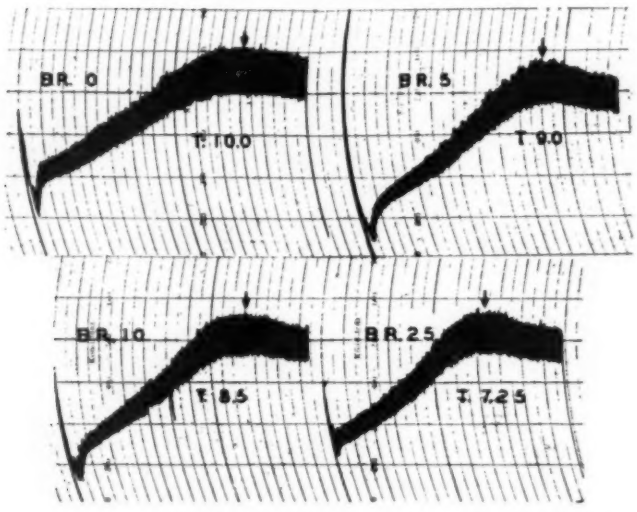

Fig. 3. Effects of bromate (BR.) on remixing time (T.) of sponge doughs.

These results showed that bromate shortens *remixing time* even in the absence of glutathione. This seems to explain the decrease in mixing time beyond that given by glutathione alone, when using both bromate and glutathione in dough. It is, however, apparent that the effect on mixing time of sponge doughs is not very significant when using normal amounts of bromate—5 mg or less per 300 g flour.

Glutathione and Bromate in Straight Doughs

Straight doughs with glutathione alone and with added bromate were mixed in the Farinograph to note mixing time effects. The results are shown in Table IV and in Figure 4.

TABLE IV

EFFECTS OF GLUTATHIONE AND BROMATE ON MIXING TIME IN STRAIGHT DOUGHS

Treatment per 300 g flour		Mixing time
Glutathione	Potassium bromate	
mg	*mg*	*min*
0	0	20.0
25	0	8.5
25	5	8.5
25	10	8.0
25	25	8.0

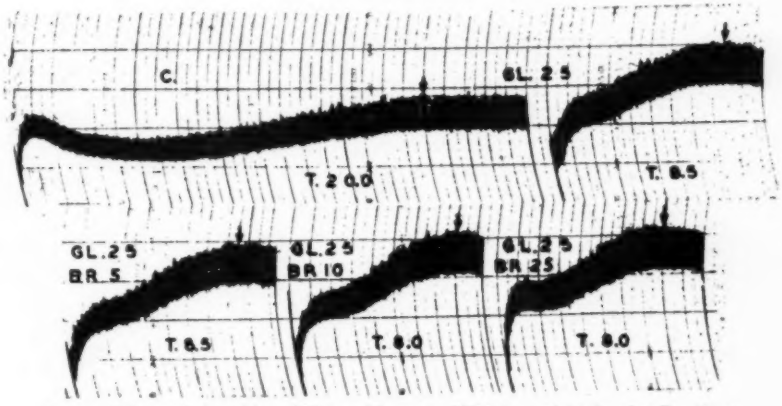

Fig. 4. Effects of glutathione (GL.) and bromate (BR.) in straight doughs; T.—time.

There was a marked reduction in mixing time because of glutathione, but the effects from added bromate under these conditions were negligible. This result was not unexpected, because it has been shown in previous work that the effects of glutathione are immediate, whereas those of bromate are delayed; the development of acidity in the dough as a result of fermentation is a factor which makes the bromate effective. The influence of bromate in straight doughs was observed, there-

fore, by remixing them in the Farinograph after a normal fermentation period; the doughs were originally mixed in the Hobart-Swanson mixer. Straight doughs with bromate and glutathione gave remixing times as shown in Table V and in Figure 5.

TABLE V

EFFECTS OF BROMATE AND GLUTATHIONE IN STRAIGHT DOUGHS, REMIXED AFTER FERMENTATION

Treatment per 300 g flour		
Glutathione	Potassium bromate	Remixing time
mg	*mg*	*min*
0	0	16.5
0	50	6.0
25	0	11.0
50	0	6.25
50	50	2.25

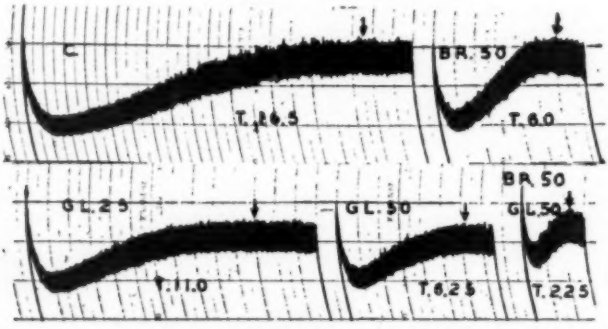

Fig. 5. Effects of bromate (BR.) and glutathione (GL.) in straight doughs remixed after fermentation; T.—Time.

Both bromate and glutathione individually produced great decreases in remixing time, but the reduction produced by their combined effects was far greater than that of either one alone. These results thus confirmed the above findings for sponge doughs.

Effects of Oxidized Glutathione and Oxidized Sodium Sulfite [1]

In view of the results thus far obtained, it appeared of interest to study the effects of oxidized glutathione; oxidized sulfite was also tried, because sulfite produces mixing effects in dough like those of glutathione.

Glutathione and sodium sulfite were therefore oxidized with potassium bromate and with hydrogen peroxide in acid solution (2 ml N/1 HCl in about 50 ml water) and then used in doughs which were mixed in the Farinograph, fermented, and then remixed in the Farinograph

[1] Oxidized sodium sulfite is, of course, sodium sulfate; the term "oxidized sulfite" is used here to emphasize the point that the product was prepared in the same manner as the oxidized glutathione.

after fermentation. The amounts of potassium bromate and hydrogen peroxide required were determined by titration and agreed with theoretical requirements. The solutions were neutralized with alkali before mixing the doughs. The results are shown in Table VI and in Figure 6. Also shown in Table VI are the results of a test in which glutathione in water solution was oxidized with iodine before use in the dough, compared to a test in which the same amounts of glutathione and iodine were added to the dough separately, so as to avoid contact between them outside of the dough.

TABLE VI

EFFECTS OF OXIDIZED GLUTATHIONE AND "OXIDIZED SULFITE" ON MIXING AND REMIXING TIME

Treatment per 300 g flour	Mixing time	Remixing time
	min	*min*
Sulfite, 10 mg, oxidized with H_2O_2	15.0	16.0
10 mg, oxidized with $KBrO_3$	15.5	15.5
Glutathione, 25 mg, oxidized with H_2O_2	7.5	10.0
25 mg, oxidized with $KBrO_3$	9.0	9.5
25 mg, oxidized with iodine	9.0	7.25
Glutathione and iodine, added to the dough separately	8.25	6.5

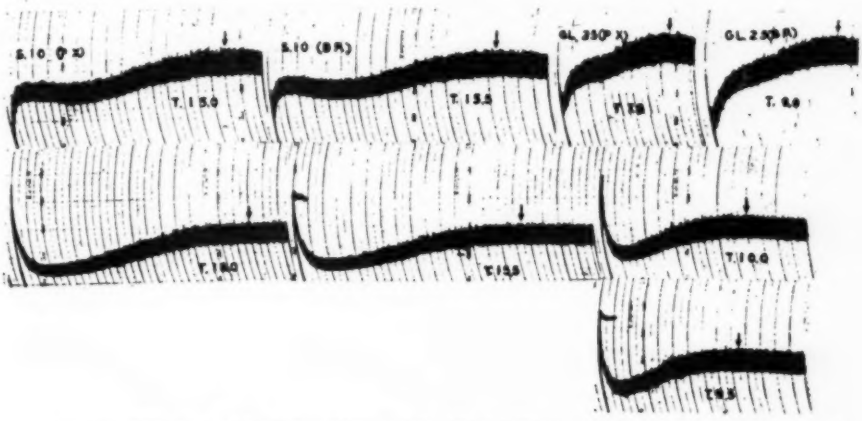

Fig. 6. Effects of oxidized glutathione and "oxidized sulfite" on mixing (four upper curves) and on remixing (four lower curves) of the same straight doughs; S.—sulfite; GL.—glutathione; PX.—oxidized with peroxide; BR.—oxidized with bromate; T.—time.

The treated sulfite acted as though it had been completely oxidized, as will be seen from the results shown. But the glutathione still produced mixing time effects similar to those of reduced glutathione.

In the following experiments, sulfite which was oxidized with iodine was compared to the same quantities of sulfite and iodine added to the dough separately; in the latter instance, direct contact between the sulfite and iodine solutions was carefully avoided before the start of dough mixing. For comparative purposes, the experiment also included a

control; sulfite alone; sulfite oxidized by exposure to air overnight; and sulfite with an excessive amount of iodine, added separately. The results are shown in Table VII and in Figures 7 and 8.

TABLE VII

EFFECTS OF SULFITE, "OXIDIZED SULFITE," AND SULFITE WITH IODINE (IN THE SAME DOUGH BUT ADDED SEPARATELY), ON DOUGH MIXING AND REMIXING TIME

Treatment per 300 g flour	Mixing time	Remixing time
	min	*min*
Control	15.5	15.0
Sulfite, 10 mg	9.5	12.5
Sulfite, 10 mg, oxidized with iodine (14.1 ml of 0.01*N*) before use in dough	14.5	14.0
Sulfite, 10 mg and iodine, 14.1 ml of 0.01*N*, added to the dough separately	10.75	6.25
Sulfite, 10 mg, oxidized by oxygen (in air)	15.5	15.0
Sulfite, 10 mg and iodine, 30 ml of 0.01*N*, added to the dough separately	11.0	2.25
Sulfate (Merck) 12 mg	15.0	15.0

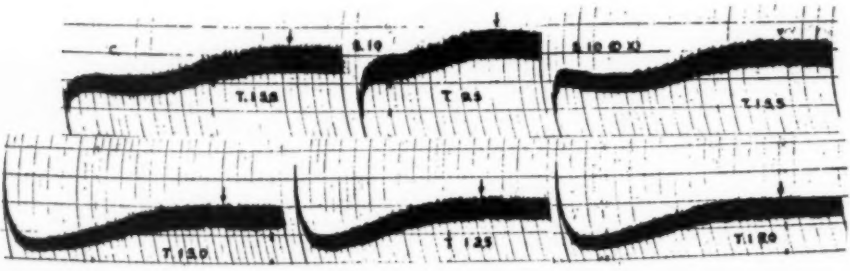

Fig. 7. Effects of sulfite and of "oxidized sulfite" in mixing and remixing; upper curves show original mixing; lower curves show the remixing of the same doughs after fermentation; S.—sulfite; OX.—oxidized in air (see Table VII); T.—time.

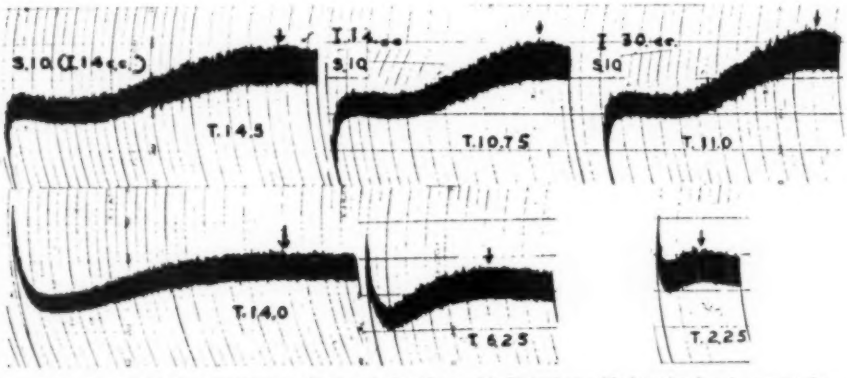

Fig. 8. Effects of "oxidized sulfite" and of sulfite and iodine when added to the dough separately, in mixing and remixing; upper curves show original mixing; lower curves show the remixing of the same doughs after fermentation; S. (I.)—sulfite, oxidized with iodine; I. S.—iodine and sulfite added to the dough separately (see Table VII); T.—time.

These results indicated (*a*) a glutathione effect resulting from sulfite in mixing, and also in remixing, though to a lesser extent; (*b*) the complete oxidation of sulfite by iodine in solution; (*c*) the failure of iodine to oxidize sulfite when they were added to the dough separately;[2] (*d*) the complete oxidation of sulfite by oxygen, and (*e*) an added reduction in remixing time owing to the cumulative effects of sulfite and iodine.

Discussion

The primary purpose of this paper is to report the results of a study of the effects of oxidizing agents and reducing matter when both are present in a normal dough formula. Our results tend to show that the improving effects of bromate are not due primarily to the oxidation of glutathione. This is contrary to the prevailing viewpoint, as expressed by Sullivan and by Ziegler. Sullivan and co-workers (1936) state that "the oxidizing agents simply change some of the S-H glutathione to the S-S form."

It is obvious that our work confirms a number of the observations made by Ziegler and other workers in the field, but our studies are not in agreement with the general conclusion reached by Ziegler, namely, that the improving effects of bromate are due mainly to the oxidation of glutathione in dough. This viewpoint is retained in his third paper, although some of the work presented there makes its validity appear doubtful.

Our most significant results showed the difference between previously oxidized sulfite in dough as compared to the effect of the same amounts of sulfite and oxidizing agent, but added to the dough batch without previous contact; in the latter instance, there was practically no oxidation of the sulfite in the dough, either during the original mixing or during the fermentation of the dough.

From this it follows that reactions between pure reagents in solution do not necessarily indicate what will happen when the same reagents are present in dough.

Since oxidizing agents overcome the harmful effects of reducing matter on loaf volume and texture without at the same time eliminating the effects of the latter on mixing time, and since there is a difference in effect between "oxidized sulfite" as compared to equivalent amounts of sulfite and oxidizing agent added separately, it is indicated (*a*) that reducing matter may not be oxidized in dough, and (*b*) that reduction and oxidation may involve independent factors in dough. Reducing

[2] This result (*c*) would represent conditions in actual baking practice, where there is no direct contact between the oxidizing agent and any reducing matter that may be present, before the dough is mixed.

control; sulfite alone; sulfite oxidized by exposure to air overnight; and sulfite with an excessive amount of iodine, added separately. The results are shown in Table VII and in Figures 7 and 8.

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	min	*min*
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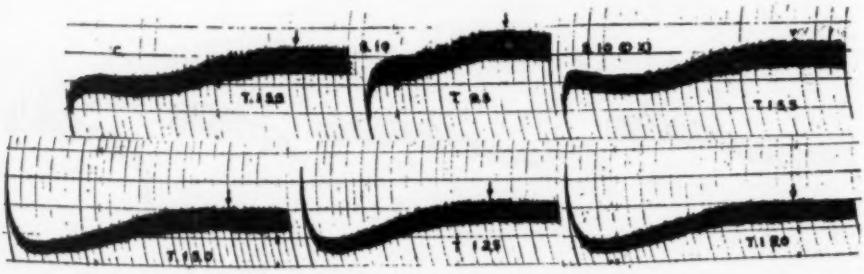

Fig. 7. Effects of sulfite and of "oxidized sulfite" in mixing and remixing; upper curves show original mixing; lower curves show the remixing of the same doughs after fermentation; S.—sulfite; OX.—oxidized in air (see Table VII); T.—time.

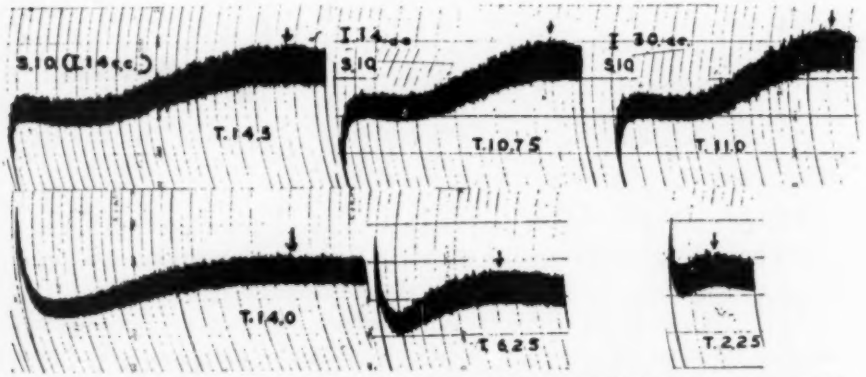

Fig. 8. Effects of "oxidized sulfite" and of sulfite and iodine when added to the dough separately. in mixing and remixing; upper curves show original mixing; lower curves show the remixing of the same doughs after fermentation; S. (I.)—sulfite, oxidized with iodine; I. S.—iodine and sulfite added to the dough separately (see Table VII); T.—time.

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[2] This result (*c*) would represent conditions in actual baking practice, where there is no direct contact between the oxidizing agent and any reducing matter that may be present, before the dough is mixed.

agents seem to act on the gluten, while oxidizing agents may act either on another part of the gluten complex, or possibly on some substance associated with it, such as the pentosans described by Baker and coworkers (1943). Reduction tends to soften the dough and make it stringy, while oxidation tightens the dough, though at a later stage in the procedure, when bromate is the oxidizing agent used; the two effects thus counteract each other, and when properly balanced will produce good volume and texture.

Reversible oxidation-reduction systems in dough are indicated (*a*) by the mixing effects of oxidized glutathione, which is possibly reduced during mixing and remixing, and (*b*) by the elimination of the "excess bromate" effect in remixing, with a decrease in mixing time, caused perhaps by the reduction of substances oxidized by bromate.

The failure of the common oxidizing agents to combine with added reducing matter in dough is not difficult to understand when it is considered that even under ideal conditions in solution these reactions are not instantaneous. In dough, opportunities for contact between these substances are greatly reduced; in addition, the presence of other oxidizable matter in dough is another factor tending to lessen opportunities for such contact.

Summary

Reducing matter, among other effects, shortened dough mixing time to a marked degree, as indicated by Farinograph curves. These effects were not eliminated by oxidizing agents, even when present in excessive amounts or in amounts sufficient to overcome the loaf volume reduction due to glutathione.

Oxidizing agents produced a decrease in mixing time after fermentation which was apparently independent of the effects of added reducing matter.

There was a marked difference between the mixing effects of "oxidized sulfite" as compared to the effects of equivalent quantities of sulfite and oxidizing agent added to the dough separately.

Oxidized glutathione was found to produce mixing effects in dough similar to those of reduced glutathione.

Reduction and oxidation probably involve different factors in dough and their effects tend to counteract each other with respect to bread quality.

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LABORATORY MALTING. IV. A GERMINATION CHAMBER FOR ROUTINE MALTING TESTS [1]

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The development of laboratory malting equipment on this continent was described by Anderson (1937) when he reported on equipment installed in the National Research Laboratories, Ottawa. This equipment was designed for examining the effect of changes in malting procedure on the evaluation of malts (Sallans and Anderson, 1939 and other papers in the series) with the object of developing a routine method for testing barleys for malting quality. Shortly after the studies at Ottawa were started, the malting equipment built at the University of Manitoba in 1927 was overhauled and details of the changes were published (Anderson and Rowland, 1937). Malting studies conducted at the University of Wisconsin (Dickson *et al*, 1942) and in Canada (Anderson, Sallans, and Meredith, 1941, and Anderson, Meredith, and Sallans, 1943 and earlier papers in these series) have now resulted in the laboratory malting test becoming a standard tool in

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barley research. Equipment for long-time use has been designed and tested in Canadian laboratories, and an improved germinator for 24 250-g samples is described in this paper.

The new germinator combines the better features of both the earlier Canadian models. It has been in operation for two and a half years in conjunction with a steep tank and kiln already described

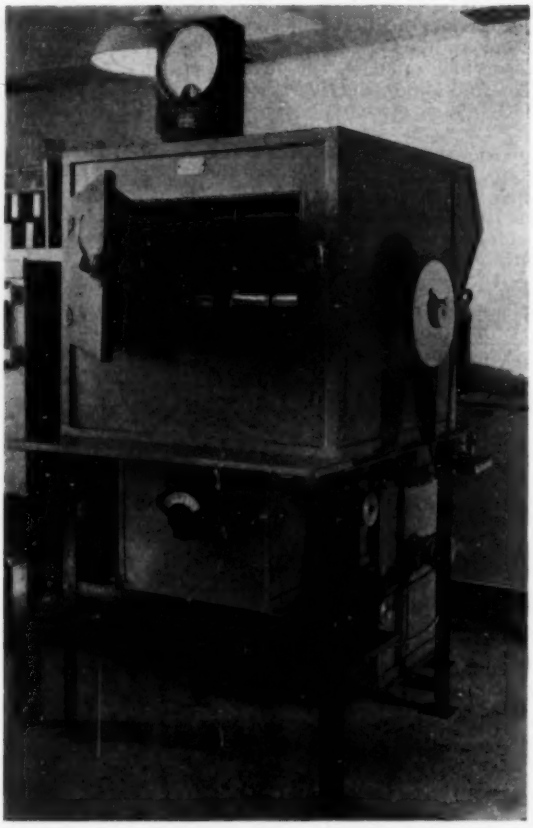

Fig. 1. Exterior view of the germinator.

(Anderson and Meredith, 1940; Anderson and Rowland, 1937). With a 6-day germination period, this equipment produces well-modified malts, with satisfactory malt yield and root loss, and it has proven satisfactory in all respects for routine studies.

Equipment and Malting Methods

A photograph of the germination unit is shown in Figure 1, and a drawing showing the main working parts is given in Figure 2. The unit

comprises essentially a sample chamber and a conditioning chamber, enclosed in a common case insulated with 4 inches of cork. The whole is protected on the outside by sheet metal and is set inside a $1\frac{1}{2}'' \times 1\frac{1}{2}'' \times \frac{1}{4}''$ welded angle-iron frame that also serves as a stand.

The sample chamber A is 28″ wide × 24″ × 24″ and has a rotating brass frame B which carries 24 sample containers. The conditioning chamber C is 15″ × 15″ × 40″ high; the lower portion D is filled with water to a depth of 10 inches, and the top 6 inches is fitted with baffles E.

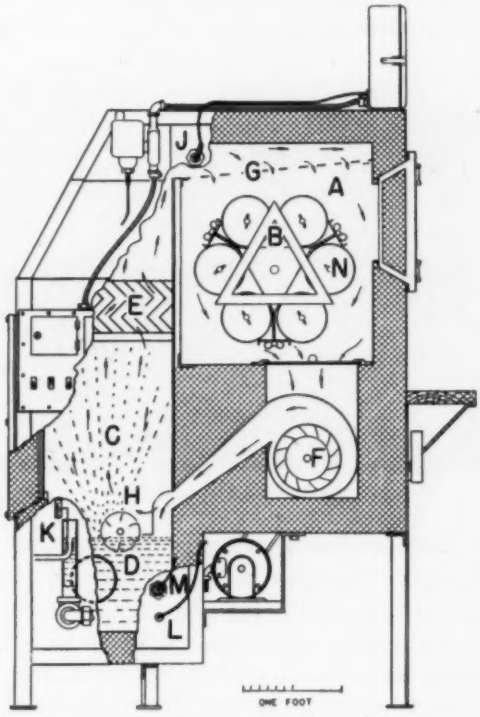

Fig. 2. Sectional view of germinator showing working parts.

Air is drawn continuously from chamber A by the fan F, passed through the spray chamber C where it is humidified, and returned through baffles E and distribution plate G into the sample chamber. The fan is set in a duct below chamber A connecting it with the spray chamber near the water level. The spray, by which air conditioning is effected, is created by a rotor shown at H. The temperature of the water in tank D is controlled by an indicating controller with its sensitive element placed in the germination chamber at J. The water level is maintained by a simple adjustable overflow device K. A dial-read-

ing thermometer, with the bulb L placed in the water tank, provides a means of estimating the relative humidity in the system, by comparing the temperature indicated by this thermometer and that indicated by the indicating controller.

A single $\frac{1}{4}$ hp motor operates both fan and spray rotor at 1100 rpm by means of V-pulleys to maintain the desired conditions. A $\frac{1}{4}$ hp SO_2 refrigeration unit, actuated by the control mentioned above, cools the water. The system is protected against freezing by an auxiliary thermal cut-out, the element of which is indicated at M. A $\frac{1}{16}$ hp motor operating through a double worm speed reducer and a chain and sprocket assembly rotates the frame B at 3 rp hour (Fig. 1).

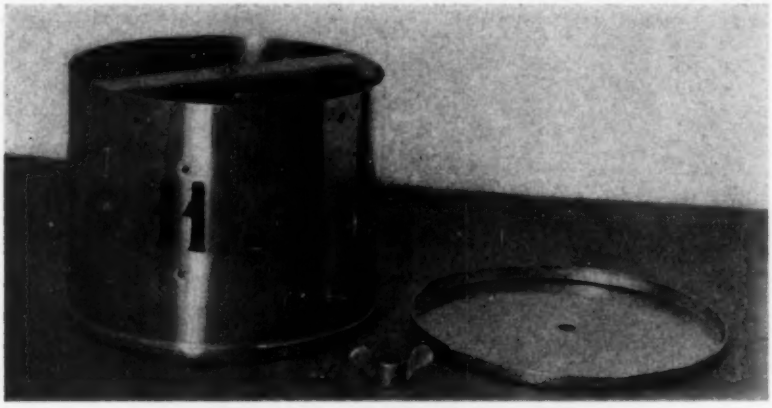

Fig. 3. Sample container.

All materials used for interior construction are corrosion resistant. The lining of the sample chamber is 24-gauge copper as are also the fan duct and baffles. The conditioning chamber is made from 16-gauge brass, brazed at the seams, with a 12″ × 16″ inspection plate at the back; the fan is also assembled from brazed brass; and the air distribution plate G is made from $\frac{1}{8}$″ brass. Shafts are stainless steel and packing glands are brass. The frame that carries the sample containers is made from angle brass; and the spray rotor consists of five $4\frac{1}{2}$″ diameter notched-and-bent disks made from 16-gauge stainless steel. All shafts pass through watertight bearings and no air enters the system except when the door is open.

The cages N in which the samples are malted are stainless-steel cylinders, 6 inches in diameter and 5 inches high, with 48 one-eighth inch holes spaced evenly over the cylindrical surface. One of these cages is shown in Figure 3. Various types of cages were tested and it was found that there was excessive loss of moisture from samples in wire mesh containers.

The unit is now operated to produce an air temperature inside the germination unit of 53°F with a relative humidity of approximately 100%. The water temperature and sample chamber temperatures are practically identical, and the capacity of the unit is such that after insertion or removal of samples, with ensuing entry of outside air, operating temperature and humidity are rapidly regained. The temperature of the malts in the cages now in use runs above that in the chamber and reaches a maximum of about 60°F on the third day following the addition of water to the samples.

Method. Under routine conditions, the malting laboratory produces 24 malts per week. The malts are made in batches of 12, and the batches are spaced alternately 3 and 4 days apart. By this procedure the steep tank and kiln, which each hold 12 samples, are in continuous use, and the germination chamber always contains two batches of different age.

The samples are steeped to 44% moisture content at 50°F (Anderson and Meredith, 1940), and then transferred to the germination chamber which is maintained at 53°F and 100% relative humidity. After 66 hr in the germinator, water is added to the samples to bring the moisture content up to 46%. The samples are then grown for three more days, making a total of six days in the germinator. They are then transferred to wire mesh cages and placed in the kiln (Anderson and Rowland, 1937) for a 48-hr kilning schedule. Roots are removed from the samples after kilning by kneading in a small bag and sifting the sample.

The system now in use to prevent matting of the samples in the germinator is a combination of the Ottawa and Manitoba procedures. The cages are rotated at 3 rp hour during the day and kept stationary for 12 hr overnight. The malts made in continuously rotating cages were similar to those produced in the Ottawa equipment, in that modification was good, but root losses were somewhat high and malt yield somewhat low. On the other hand, when the cages were stationary, the malts were similar to the original University of Manitoba malts; root losses were very low, malt yield was very high, and there was considerable undermodification.

Precision. The germinator has been in use for two and a half years for testing plant breeders' varieties and other laboratory samples. Malts that are satisfactory in modification have been produced from the malting varieties and the reproducibility of the equipment, as measured by differences between duplicate samples, is adequate. The standard deviations for the mean of duplicates for a number of routine investigations carried out in the laboratory are given in Table I.

TABLE I

STANDARD DEVIATIONS FOR MEANS OF DUPLICATES FOR MALT PROPERTIES

Project	No. of varieties	No. of stations	D.F.	Malt yield	Roots	Saccharifying activity	Extract	Wort N.
				%	%	°L	%	%
U. of Alberta	4	3	12	0.14	0.14	1.6	0.12	0.007
Sask. Regional	6	3	18	0.22	0.20	2.1	0.14	0.014
Ont. Regional	4	12	45	0.32	0.25	3.2	0.17	0.019
National Trials	6	5	30	0.21	0.14	1.6	0.17	0.010

A further measure of the reproducibility of the equipment was obtained by making four consecutive replicate batches of malt—preceded and followed by blank batches—from samples of two barleys. The mean values for the malting and malt properties of each batch are given in Table II. The analyses of variance of the data are given in Table III.

TABLE II

MEAN VALUES FOR MALTING AND MALT PROPERTIES FOR EACH BATCH

Property	Batch			
	I	II	III	IV
Weight after germination, g	461.4	460.1	461.7	460.2
Malt yield, %	91.4	91.6	91.5	91.4
Roots, %	4.4	4.3	4.5	4.3
Saccharifying activity, °L	115.7	110.9	110.3	108.5
Extract, %	75.3	75.4	75.3	75.3
Wort nitrogen, %	1.07	1.07	1.06	1.08
Index of nitrogen modification, %	37.2	37.2	36.7	37.6

TABLE III

MEAN SQUARES FOR VARIOUS PROPERTIES

Property	Mean squares			
	Batches	Samples	Ba.×sam.	Within ba.
Weight after germination, g	2.083	0.080	0.307	0.850
Malt yield, %	0.163	0.010	0.277**	0.030
Roots, %	0.083*	0.001	0.003	0.025
Saccharifying activity, °L	114.8**	225.3**	4.2	7.9
Extract, %	0.037	0.227	0.023	0.021
Wort nitrogen, %	0.0012	0.0019	0.0007	0.0005
Index of nitrogen modification, %	1.554	1.172	0.731	0.530
Degrees of freedom	3	1	3	40

* Denotes significantly greater than mean square due to error within batches. Single signs denote that 5% level of significance is attained. Double signs denote that 1% level of significance is attained.

Differences between batches are very small, except with respect to saccharifying activity, and the interaction errors between batches and samples and the errors within batches are small. The variations in saccharifying activity may be attributed to kilning; saccharifying activity is considerably more sensitive to deviation in kilning temperature than to changes in germination conditions.

Discussion

The faults and virtues of the equipment originally installed at the University of Manitoba and in the National Research Laboratories, Ottawa, were taken into account in designing the new germinator. The unit resembles the Ottawa equipment in that the air is circulated continuously and is conditioned by means of water sprayed from a controlled bath by a notched-disk rotor, and sample cages are clamped on a rotating frame. It resembles the Winnipeg equipment in that the conditioning chamber and germination chamber are one unit, which minimizes the rise in air temperature between spray and germination chambers and thus facilitates the maintenance of high relative humidity in the latter.

The equipment has sufficient flexibility in the control of temperature, humidity, and air speed to permit studies of malting procedures. An important feature is that there is no bleed-in of air, so that the carbon dioxide content of the air in the chamber is increased by the respiration of the growing samples. When the equipment was built, it was the intention to install an auxiliary carbon dioxide supply, with an automatic control in the chamber, to provide for studies of the effect of malting at different carbon dioxide levels. This development was prevented by the war, but will be kept in mind for future consideration.

The equipment is now installed in the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, together with the steep tank described by Anderson and Meredith (1940), and the kiln described by Anderson and Rowland (1937). These three units are characterized by simplicity of design and sturdy construction, but incorporate many automatic features. They provide the equipment required for routine laboratory malting of 24 samples per week. The malting method has been designed to produce malts similar to those made in commercial plants, and to evaluate the malting quality of new varieties of barley in comparison with the standard variety, O.A.C. 21. The equipment and method fulfill these conditions admirably, and provide a standard malting test of adequate precision for all routine studies.

Summary

A laboratory germinator with a capacity for growing twenty-four 250-g malts is described. It consists of a sample chamber and a conditioning chamber, enclosed by a common insulated case. Air is circulated continuously through the two chambers. It is humidified and cooled in the conditioning chamber, and maintains conditions of 53°F at saturation within close limits in the sample chamber. Samples are grown in cylindrical containers clamped on a rotating frame. The unit has been in use for over two years, and produces well-modified malts with adequate reproducibility.

Acknowledgments

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STORED AND DAMAGED WHEATS FOR STARCH PRODUCTION

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Since about 1941 there has been a tremendous increase in corn utilized by the corn wet-milling industry in the United States. Approximately 75 million bushels annually are normally used by wet

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millers for the production of starch, dextrins, corn sirup, corn sugar, and by-products. In 1942, wet millers ground 130 million bushels. The increased demands for starches and glucose have arisen because of the large quantities required for lend-lease purposes and for essential war industries, and to the virtual cessation of importation of tropical starches. Even though the starch industry has been operating at full capacity, it has not been able to satisfy all the demands for its products. Voluntary rationing of starches, sirups, and other products has therefore been necessary.

The industry has faced the additional problem of an uncertain source of raw material. Grain consumption and distribution have been affected by a combination of war measures leading to a shortage of corn on the cash market. The estimated disappearance of corn for feeding and industrial use during the 1942–43 crop year exceeded the 1943 crop production by more than 175 million bushels. The deficit is being inadequately met by drawing on the carry-over and by rationing to essential users. No relaxation in the demand for corn can be foreseen over the next few years. Exhaustion of the carry-over and a shortage of corn must result unless steps are taken to equalize corn production and disappearance.

Producers or prospective producers of starch or sirups may have to use wheat and other small grains as raw material. Unfortunately, these grains are scarcely more plentiful than corn. Because of the preeminent position of wheat as a food and the possible need of exporting large quantities to stricken parts of the world, as well as its recently attained position as an industrial raw material, a liberal stockpile of wheat is a necessity. From the standpoint of economy of foodstuffs, it would therefore seem desirable to obtain starch, whenever possible, from such wheat and other grains as cannot advantageously be used for food or feed.

Although grain stored under the most favorable conditions may retain its milling and baking characteristics for several years, storage for a longer time or under less favorable conditions will not only lower its milling value but also decrease its worth for food or feeding (Jones and Gersdorff, 1941). As of March 31, 1943, there were about 52 million bushels of corn and approximately 245 million bushels of wheat from the crop years of 1938 to 1942 inclusive, owned by the Commodity Credit Corporation. By December 31, these amounts had been reduced to 0 and 116 million bushels, respectively. During this period, all of the aged grain stored on farms, on loan, had been called in, and much of it had been sold. Some of the grain held by the Commodity Credit Corporation, as well as much of that stored privately on farms, may serve as feed, but movement of stored grain to industry, with

consequent release of all current crops for food and feeding, would be preferable from the nutritional standpoint.

Weather, disease, or insects may cause more damage to grain in the field that it would suffer in storage. At marketing centers, approximately 2 to 24% of the wheat inspected is put into "sample grade" because of some type of damage. The amount varies with season and locality. It was conservatively estimated in June 1943 that 2.5% of the wheat on hand in this country was of sample grade. Although this percentage was low, it amounted to 20 million bushels, a quantity which would not be negligible if made available for starch production.

Stored and damaged grains, especially wheat no longer suitable for food or feeding, appear to be logical supplements of corn for the production of starch and starch derivatives. Little or no information is available, however, as to processing difficulties which might be expected. Knowledge concerning the properties of starches from stored and damaged wheats is equally scanty. Studies reported below were therefore carried out on representative unstored sound, stored sound, and disease-, weather-, and heat-damaged wheats, to determine comparative ease of processing, and to characterize the starches obtained. These wheats will hereafter be referred to as "sound," "stored," and "damaged," respectively.

Experimental

Samples of stored wheat from all known sources and all available types of damaged wheat were obtained in an attempt to get material as old and as severely damaged as possible. Sound, stored, and damaged wheats were obtained from state experiment stations in this country and from Canada. Additional samples of stored and damaged wheats were furnished by the Commodity Credit Corporation and the Office of Distribution of the War Food Administration. The sound wheats included Chiefkan, Kawvale, Triplet, Rex, and Hymar which represent types of wheat that, because of relatively poor milling or baking characteristics or because of the area in which they are grown, are often produced in excess of demand.

Extraction Methods. The wheat was thoroughly cleaned before processing, but care was taken not to lessen the degree of damage in stored or damaged wheat samples. The whole grain, rather than flour, was used as raw material for starch extraction. This obviated the necessity for milling quantitatively a large number of small samples. Preliminary experiments had shown that comparable results were obtained when whole wheat and flour were processed.

Laboratory processing methods involving the steeping of wheat, either in water or in a weak solution of sulfur dioxide in water, were

used. Preliminary experiments showed that starch could be more easily extracted after the sulfur dioxide steep. The water steep was used as the basic extraction method, however, since it more nearly simulated commercial wheat starch extraction conditions and avoided possible removal of undesirable odor or color from damaged grain by the sulfur dioxide. A water steep also eliminated the possibility of action on the starch by sulfur dioxide. The acid steep was used for comparative purposes or to process wheats that did not give good yields or white products after a water steep.

The two laboratory processing methods used are described under (1) and (2) below.

(1) A weighed amount of wheat, generally about 400 g, was steeped in cold distilled water at approximately 4°C for 24 hr, ground twice through a Russwin No. 2 handmill with nut-butter cutter, after which it was made into a stiff dough with distilled water and allowed to stand 1 hr at room temperature to permit hydration of the gluten. Starch was then removed by working the more or less coherent mass in successive portions of distilled water. All extraction liquors were combined and worked on No. 17 standard bolting silk to remove pieces of bran and the larger gluten particles with which the starch was contaminated. The partially cleaned starch was recovered from suspension by centrifuging. Finely divided gluten was removed by scraping away the upper layer which formed during centrifuging. This layer also contained small starch granules and, in general, corresponded to the material passing over the end of a commercial starch table. The starch was resuspended in distilled water, centrifuged, and the gluten and other light matter scraped away as before; several repetitions of this procedure yielded a white starch with minimum contamination, which was dried in a mechanical convection oven at 44°C to a moisture content of about 12–15%.

(2) A weighed amount of wheat was steeped in a glass container in a water bath at 44°C for 24 hr in distilled water or in SO_2 solution of known concentration, usually 0.20%. The steep water was continually circulated by a centrifugal pump. At the end of the steeping period, the steep liquor was decanted and the wheat ground in the same manner as described under (1). The ground material was immediately suspended in distilled water and worked on No. 17 standard bolting silk. From this point on, the procedure was the same as described under (1). This is essentially the method described by Slotter and Langford (1943).

Prime-quality starch is obtained by both of these methods. Both methods give recoveries approximating those obtained industrially from wheat.

Analytical Methods. Wheat moisture contents were determined by drying the ground grain for 1½ hr at 130°C in a Brabender moisture tester. Moisture of starch was determined by drying a 0.2-g sample to constant weight over P_2O_5 at 100°C in an Abderhalden drier. Percentage of starch in wheat was determined by a diastase method (Association of Official Agricultural Chemists, 1940). Nitrogen was determined by the Kjeldahl-Gunning-Arnold method (A.O.A.C., 1940) with the modification that the ammonia was received in boric acid solution (Winkler, 1913). The ash content was determined by incinerating the starch at 700°C for 3 hr.

Viscometric Methods. Viscosity measurements were made in a Stormer viscometer, using the 100-g weight. Determinations were made at 90°C, using either 2 or 3.5% suspensions of starch in distilled water pasted for 10 min in a boiling-water bath. Eleven readings were taken on each sample, the first immediately after the paste was placed in the viscometer cup, the others at 1-min intervals thereafter. No change was observed during this period when 2% pastes were used. The viscosity of 3.5% pastes decreased, however, during successive determinations; for this concentration the first and eleventh readings are recorded.

TABLE I

DATA ON EXTRACTION AND PROPERTIES OF STARCH FROM SOUND WHEAT

(All data given on moisture-free basis)

Wheat					Starch					
Type	Variety	Origin	Starch content	Steep used	Recovery	Nitrogen	Ash	Relative viscosity of paste		
								2%	3.5%	
									Initial	10-min
			%		%	%	%			
Soft red winter	Mixture	Commercial	66.7	Cold water	80	0.04	0.16	1.4	3.7	3.3
Soft red winter	Trumbull	Wooster, Ohio	67.5	Cold water	75	0.04	0.14	1.4	3.5	3.0
Soft white (winter)	Rex	Colfax, Wash.	69.7	0.2% SO_2	77	0.04	0.09	1.3	2.3	2.2
			69.7	Cold water	76	0.04	0.14	1.3	2.3	2.2
Semihard red winter	Kawvale	Topeka, Kansas	63.8	0.2% SO_2	75	0.05	0.15	1.3	3.5	2.7
			63.8	Cold water	70	0.04	0.16	1.4	2.7	2.4
Semihard red winter (Western red)	Triplet	Colfax, Wash.	69.9	0.2% SO_2	78	0.05	0.15	1.3	2.6	2.6
			69.9	Cold water	75	0.04	0.15	1.3	2.3	2.1
Hard red winter	Turkey	Minneapolis, Kansas	65.7	0.2% SO_2	80	0.05	0.17	1.4	3.5	2.8
Hard red spring	Thatcher	Bozeman, Mont., 1941	65.0	Cold water	75	0.04	0.14	1.4	3.1	2.5
		Bozeman, Mont., 1942	66.4	Cold water	80	0.04	0.17	1.3	2.7	2.3
Hard red winter	Chiefkan	Wamego, Kans.	65.5	Cold water	73	0.04	0.13	1.4	2.7	2.4
			65.5	0.2% SO_2	77	0.04	0.08	1.3	2.4	2.3
White Club	Hymar	Colfax, Wash.	70.1	0.2% SO_2	73	0.03	0.10	1.3	2.4	2.3
			70.1	Cold water	72	0.04	0.13	1.3	2.4	2.3

Processing of Sound Wheat. As a basis for comparison, starches were extracted from several dozen sound hard and soft wheats shortly after harvesting. Representative data are given in Table I. Extractions by both methods are included to furnish a basis for comparison with data in other tables and to show how closely results obtained by the two methods agree. Use of method (1) is indicated in the tables by the words "cold water" in the column headed "steep used."

Each laboratory method yielded a satisfactory product. Recovery of starch usually ran between 75 and 80%. Nitrogen and ash were as low as or lower than the amounts found in commercial wheat starches. In all cases, the starches were white and had no abnormal odors. Viscosities were slightly (0.5 to 1.0 sec) higher than those of commercial wheat starches.

Processing of Stored Wheat. About two dozen different samples of wheat, stored (1) under laboratory conditions, (2) in Commodity Credit Corporation bins, or (3) on the farm, were processed in 1943 for starch. All samples were reported to be sound when placed in storage and none had become damaged while stored. Representative data are summarized in Table II.

TABLE II

DATA ON EXTRACTION AND PROPERTIES OF STARCH FROM STORED WHEAT

(All data given on moisture-free basis)

Wheat					Starch					
Variety	Source	Crop year	Starch content	Steep used	Recovery	Nitrogen	Ash	Relative viscosity of paste		
								2%	3.5%	
									Initial	10-min
			%		%	%	%			
Thatcher	C.C.C. bin, Jamestown, No. Dakota	1941	62.7	Cold water	75	0.05	0.12	1.3	3.1	2.6
Marquis		1940	61.0	Cold water	80	0.05	0.17	1.4	3.4	2.7
	Paskweign, Saskatchewan [1]	1928	61.7	Cold water	65	0.04	0.15	1.4	3.4	3.0
	Bidora, Saskatchewan [1]	1928	64.1	Cold water	65	0.04	0.18	1.3	2.9	2.6
	Saskatoon, Saskatchewan [1]	1928	61.0	Cold water	70	0.04	0.13	1.4	3.2	2.8
Turkey	Clayton, Kansas, farm storage	1927	68.4	Cold water	65	0.05	0.17	1.3	2.6	2.4

[1] These samples were stored in the laboratory at Saskatoon, Saskatchewan, and supplied to us through the cooperation and courtesy of Dr. A. G. McCalla, University of Alberta, and Dr. R. K. Larmour, University of Saskatchewan.

Even after 14 to 15 years of farm or laboratory storage, wheat gave a good yield of starch. Recovery tended to be somewhat lower from old wheat but was still within the range obtained for new wheats. The lower recovery may have been due to peculiarities of the samples

other than those caused by storage. Available data on the wheats as they entered storage are not sufficient to elucidate this point. The nitrogen and ash contents and paste viscosity of starch from stored wheat were similar to those from new, sound wheat (*cf.* Tables I and II).

Processing of Damaged Wheat. More than three dozen samples of damaged wheat were processed for starch. These included musty and sour wheats as well as those injured by weathering, frost, scab and blight, weevil and mold, sprouting, dead germ, incipient heating, and heat. Data on representative samples are given in Table III.

TABLE III

DATA ON EXTRACTION AND PROPERTIES OF STARCH FROM DAMAGED WHEAT
(All data given on moisture-free basis)

Wheat			Starch					
Type of damage	Starch content	Steep used	Recovery	Nitrogen	Ash	Relative viscosity of paste		
						2%	3.5%	
							Initial	10-min
	%		%	%	%			
Frost damage, 85%	67.0	0.2% SO_2	70[1]	0.07	0.11	1.3	3.0	3.0
Very musty	65.1	Warm water	80	0.04	0.14	1.4	3.2	3.2
Weathered in field 8 weeks	64.0	Cold water	80[1]	0.05	0.13	1.4	3.5	2.7
Scab and blight, 14% (Graded No. 5)	61.3	Cold water	80	0.04	0.16	1.4	3.5	2.8
Weevil and mold, 40%, sour odor	60.0	0.2% SO_2	80[1]	0.05	0.08	1.4	3.1	2.7
Sprout damage and incipient heat damage, 58%	65.2	0.2% SO_2	75[1]	0.05	0.14	1.4	4.1	3.2
Dead germ, 39%; heat damage, 26%	64.1	0.2% SO_2	50[1]	0.08	0.12	1.3	2.6	2.4
Mold and slight heat damage, 49%; weevil, 4%	64.2	0.2% SO_2	80[1]	0.04	0.12	1.4	2.9	2.6
Heat damage, 57%; incipient heat damage, 31%; musty odor	63.4	0.2% SO_2	65[1]	0.16	0.15	1.2	2.0	1.8
Heat damage, 79%; incipient heat damage, 14%	66.0	Cold water	70[1]	0.05	0.16	1.4	3.3	2.8

[1] A slightly musty or grassy odor was observed after the starch had been kept in a closed container for several days.

Several of the damaged wheats gave off-color starch when steeped in cold water. In many instances, separation of protein from the starch was difficult. These wheats were therefore processed with a sulfur dioxide steep, as indicated in the table. Not only was the color of the final product improved, but separation of the starch and protein was facilitated. In the case of one heat-damaged wheat, however, considerable difficulty was experienced in removing the gluten from the starch. As a consequence, the final product had a high nitrogen content (0.16%).

Starch was obtained in fair-to-good yield from all samples of damaged wheat studied. Yields were normal from most samples, but low from heat-damaged wheat and from that having dead germ.

All starches were white except those from two badly heat-damaged wheats, which were pale tan, perhaps due to the presence of humins. One of these wheat samples also had dead germ.

Several of the extracted starches had faint unpleasant odors. The odor was noticed after the starch had been kept for several days in airtight containers. Had the starch been dried at a higher temperature and stored in cloth bags, as in commercial practice, practically no odor would be expected. The hot starch pastes had slightly more odor than the unpasted starches.

In general, nitrogen and ash contents as well as paste viscosities were within the limits of those observed for starch from sound wheat (*cf.* Tables I and III). As already mentioned, one starch had high nitrogen content; this starch also had low viscosity.

Microscopically, starch from damaged wheat could not be distinguished from starch prepared from sound wheat. The granules were birefringent and exhibited the typical maltese cross when examined between crossed nicols. The gelatinization temperatures were within the normal range of those from sound wheats.

Discussion

The experimental data suggest that starches from stored and damaged wheats, excepting those badly heat-damaged or having dead germs, are unchanged and are therefore suitable for industrial use. Although the diversion of stored and damaged wheat to industry would be advantageous from a nutritional viewpoint, it must be recognized that certain factors complicate such a program.

Wheat is stored throughout a wide area in this country. Severe damage likewise is rarely confined to one locality. Accumulation of stored and damaged stocks at the point of processing therefore presents a collection problem.

Dry and possibly wet milling of some damaged wheat would require cleaning of the mill before it could be used for sound grains. Consequent loss of time could be justified only if the mill could operate for a considerable length of time on damaged wheat. In other words, only relatively small mills could afford to handle this grain.

By-products from stored and damaged grain would be expected to be inferior to those from sound wheat. Sourness and mustiness, for example, might remain in the bran and decrease its feed value. The effects of various types of grain damage upon gluten are not known, but storage is reported to have an unfavorable effect upon the value of wheat gluten for food and feed (Jones and Gersdorff, 1941). There is little doubt, however, but that the by-products could be used for other

purposes. Some of these factors would be compensated for by the appreciable discount in price of sample-grade wheat.

For some industrial purposes, grains are utilized for their starch content without the necessity for mechanical separation of the starch. Some cereal flours, for example, are pregelatinized for use as foundry core binders. Whole grain and wheat grits are now used by distilleries for the production of industrial alcohol. Such industries are also outlets for most types of damaged grain in which the starch is sound.

Summary

Wheat stored for 3 to 15 years in a cool, dry, well-ventilated place in the laboratory or in farm bins is suitable for starch production. Recovery of starch seems to decrease somewhat as the wheat ages. So far as can be determined, the properties of starch are not altered by aging of wheat.

Common types of damage to wheat, other than severe heating, do not render it unfit for starch production. A few types of damaged wheat yield starch having a slight off-odor. Only in badly heat-damaged wheat were the properties of starch found to be modified.

Stored wheats and most damaged wheats appear to be suitable for industrial uses.

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THE AMYLOLYTIC AND PROTEOLYTIC ACTIVITY OF SOYBEAN SEED

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In 1933 Orestano reported that soybean seed contains only beta-amylase. Recently, Newton and co-workers (1939, 1943) observed that the amylolytic activity of soybeans decreases slightly during germination and that it consists mainly of beta-amylase. These workers also described a method for preparing enzyme concentrates.

There is no extensive literature on the proteinase of soybeans. Blagowestschenski and Melamed (1934) found that the soybean contains proteolytic enzymes which digest casein and peptone at pH 5.0.

The investigations recorded in this paper deal with the amylolytic and proteolytic enzymes of soybeans and of germinated soybeans. These enzymes have their own important intrinsic commercial value as does the soybean itself. It is therefore desirable to know more about the enzyme systems involved and their respective activities.

Amylase Studies

Materials and Germination Procedure. Several varieties of soybeans and other grains were used in these studies. For germination, the seeds were soaked in water for 24 hr at 15°C and then grown for 6 and 12 days respectively at 15°C. Precautions were taken to hold bacterial and fungal growth to a minimum, and samples that did become infected were discarded. After germination, the samples were dried in vacuum at room temperature, and in the case of soybeans the material was defatted with petroleum ether.

Preparation of Extracts for Determination of Amylase Activity. For these tests the finely ground materials, and in the case of soybeans the defatted beans, were extracted with 20 parts of distilled water for 2½ hr at 20°C, shaking the mixture occasionally. Simultaneous tests were carried out, using papain-cysteine in the extraction for the liberation of the bound amylase according to the technique recommended by Snider (1941). In preparing the amylase concentrates according to Newton *et al* (1939), 250 g of the finely ground seeds and 750 ml of 30% ethanol were placed in a 2-liter flask. The mixture was gently agitated for 10 min in a shaking machine. Then it was pressed through toweling and centrifuged. To the clear fluid, two volumes of ethanol were added. The precipitate was centrifuged off, and in the case of soy-

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beans the yellow color was removed by washing twice with 30 ml portions of ethyl ether. The precipitates were dried in vacuum at room temperature.

Methods. The methods developed by Sandstedt, Kneen, and Blish (1939) and Kneen and Sandstedt (1941) for measuring the amylase activities of malt were employed in these studies.

Saccharogenic Activity (Kneen and Sandstedt, 1941). The degree of saccharification occurring in 15 min was determined, using 20 ml of 2% Lintner starch buffered with acetate buffer to pH 4.7, plus extract from the material tested and sufficient water to give a total volume of 30 ml. *Saccharogenic units* were calculated as the number of g of soluble starch converted to maltose in one hr at 30°C by one g of dry material tested. As indicated in the method, a starch-extract "blank" was also run, and precaution was taken not to exceed 40% conversion of starch to maltose.

Alpha-Dextrinogenic (Alpha-Amylase) Activity (Sandstedt, Kneen, and Blish, 1939). The rate of dextrinization of Lintner starch, buffered with acetate buffer to pH 4.7, was measured in the presence of an excess of beta-amylase, as indicated by the appearance of a "red-brown" color which the reaction mixtures gives with iodine. *Alpha-amylase units* were calculated as the number of g of soluble starch which, under the influence of an excess of beta-amylase, are dextrinized in one hr at 30°C, by one g of the dry material tested. Sandstedt and co-workers found in numerous determinations on malt that the presence of an excess of beta-amylase in this test causes the rate of dextrinization to be 5.4 times as rapid as it would be when the same amount of alpha-amylase acted alone. Hence a "true" measure of the alpha-dextrinogenic activity of malt would be secured by dividing the alpha-amylase units by 5.4.

Beta-Saccharogenic (Beta-Amylase) Activity. This activity was calculated from the saccharogenic and alpha-dextrinogenic activities according to the method of Kneen and Sandstedt (1941) and expressed as *beta-amylase units* indicating the number of g of soluble starch converted to maltose in one hr at 30°C by the beta-amylase of one g of dry material tested. Kneen and associates have found on a number of malts tested that the degree of saccharification, due to beta-amylase alone, ranges from 74 to 94% with an average value of 84%. The remaining saccharification was found to be due to alpha-amylase. It is obvious that where alpha-amylase is lacking, the saccharogenic activity may be considered as being due entirely to beta-amylase.

Dextrinogenic Activity (Kneen, Beckord, and Sandstedt, 1941). This activity was determined under the same conditions used for alpha-dextrinogenic activity with the exception that the addition of an excess

of beta-amylase was omitted. *Dextrinogenic units* were calculated as the number of g of soluble starch dextrinized in one hr at 30°C by one g of dry material tested.

TABLE I

AMYLOLYTIC ACTIVITY OF SOYBEAN AS COMPARED WITH BARLEY AND WHEAT

Exp. No.	Grain	Saccharogenic activity	Alpha-dextrinogenic (Alpha-amylase) activity	Beta-saccharogenic (Beta-amylase) activity	Dextrinogenic activity
		units	*units*	*units*	*units*
1	Soybean A	15.7	trace	15.7	trace
2	Soybean A (Germinated 6 days)	16.3	trace	16.3	trace
3	Soybean A (Germinated 12 days)	17.5	trace	17.5	trace
4	Barley	7.5	trace	7.5	trace
5	Wheat	6.4	trace	6.4	trace
6	Soybean concentrate	201.5	6.4	201.0	6.8
7	Barley concentrate	210.9	4.8	210.7	4.6
8	Wheat concentrate	235.6	4.3	235.4	4.4

Discussion of Results. Table I presents values obtained for amylolytic activity of ungerminated and germinated soybeans, and also for a concentrated soybean extract. For comparison, similar determinations were made on barley, wheat, and concentrated extracts from barley and wheat. There is no published method available for measuring very small amounts of alpha-amylase such as may be present in ungerminated grain. The methods employed in the present study for measuring alpha-dextrinogenic activity and dextrinogenic activity gave results which confirm the findings of other workers to the effect that soybeans, like barley or wheat, contain very little, if any, alpha-amylase. However, it was found that germination does not increase either the alpha-amylase or the dextrinogenic activity of soybeans, and in this respect soybeans differ from barley and wheat. In view of the fact that alpha-amylase is practically absent, the saccharifying activity of soybeans appears to be entirely due to beta-amylase; hence, beta-saccharogenic activity is identical in this case with saccharogenic activity.

The soybean concentrate manifests amylase activities similar to that of barley and wheat concentrates. The dextrinogenic and alpha-dextrinogenic activities of the concentrates were measurable and were found to have almost identical values, which was due to the fact that both were determined in the presence of an excess of beta-amylase. However, we are inclined to assume that this measurable rate of dextrinization found in the concentrates is caused rather by the saccharifying activity than by the dextrinizing activity of the concen-

trated extracts. In other words, the alpha-amylase is also here present only in traces and could not be estimated by the methods used in this study. In view of the fact that alpha-amylase activity was found to be negligible, under the conditions tested, the saccharogenic activities of the concentrates were also practically identical with their respective beta-amylase activities.

The saccharogenic activity was also determined on extracts prepared with water and with papain-cysteine from ungerminated and germinated soybeans, and compared with those prepared from ungerminated and germinated barley and wheat. The results of these tests are recorded in Table II. It will be noted that, in contrast to barley and

TABLE II

THE EFFECT OF PAPAIN-CYSTEINE ON SACCHAROGENIC ACTIVITY

(pH 4.7—Acetate buffer)

Exp. No.	Nature of material	Saccharogenic units	
		Extraction with water	Extraction with papain-cysteine
1	Soybean A	15.7	16.4
2	Soybean A, germinated 6 days	16.8	16.6
3	Soybean B	13.2	13.4
4	Soybean B, germinated 6 days	13.8	13.8
5	Barley A	7.5	17.6
6	Barley A, germinated 6 days	18.0	24.7
7	Barley B	5.2	13.6
8	Barley B, germinated 6 days	14.2	19.2
9	Barley C	4.6	13.1
10	Barley C, germinated 6 days	14.4	19.5
11	Wheat A	6.0	20.1
12	Wheat A, germinated 6 days	16.8	24.7
13	Wheat B	6.4	19.6
14	Wheat B, germinated 6 days	12.9	20.7

wheat, soybeans showed very little change in saccharogenic activity either in the course of germination or due to the action of proteolytic enzymes. In this connection it is assumed that the proteolytic enzymes present in the soybean itself exert little action on liberation of amylase in the course of 2½ hr of extraction with water at 20°C. When the results obtained for the water extracts and for the papain-cysteine extracts from ungerminated soybeans are compared with those secured from ungerminated barley and wheat, it is apparent that the beta-amylase present in soybeans is in a "free" state, while that present in barley and wheat is largely in a "bound" state, and is released from this bound state by the papain-cysteine action.

The increase in saccharogenic activity of barley and wheat during germination is due not only to the liberation of bound beta-amylase, but also to the development of alpha-amylase.

The optimum pH range for saccharogenic activity of the soybean was found to be between pH 5.18 and 6.38 with an apparent maximum value close to pH 5.9 (Fig. 1).

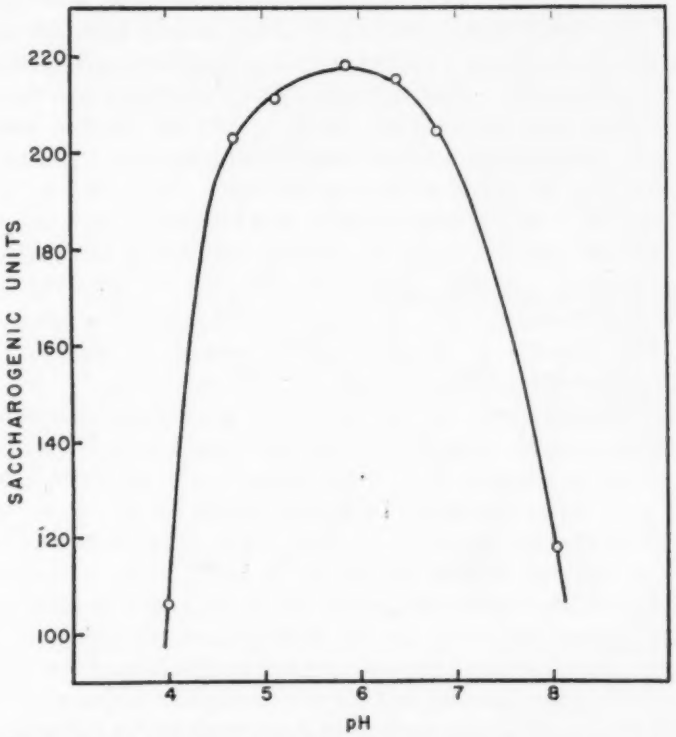

Fig. 1. Effect of pH on saccharogenic activity of soybean amylase concentrates. Saccharogenic units: g of soluble starch converted to maltose in 1 hr at 30°C by 1 g of concentrate.

Judging from the limited number of samples tested, the potential or total saccharogenic activity of soybeans appears to be of the same order as that of barley or wheat; however, since all of the soybean beta-amylase is free, a simple water extract shows much more saccharogenic activity. For convenience, these comparative tests were made at pH 4.7, which is the optimum for barley and wheat amylase but considerably below the optimum for soybean amylase. If determined at the latter pH (5.9), the values for saccharogenic activity of soybeans would be increased by 8–10%.

Protease Studies

Method. In measuring the proteolytic activity of the soybean extracts, a method was employed that had been used successfully in this laboratory in several studies of a similar nature. The method was

essentially the Willstätter (1926) method, using an alkali titration of carboxyl groups in alcoholic solution as modified by Linderström-Lang and Sato (1929). In preparing the proteolytic digest, 10 ml of either a 12% gelatin solution or a 6% casein solution which had been adjusted to pH 7 with citrate buffer, and 10 ml of the extract from the samples were mixed thoroughly in a corked test tube, both solutions having been attempered to 37.5°C. Two drops of toluene were added to the tubes, and they were then digested for 24 hr at 37.5°C. Before and after incubation, 2-ml aliquots were pipetted from each mixture to a titration flask containing 10 ml of 95% ethanol which was sufficient to stop enzyme action. The alcohol mixture was titrated in the presence of 0.1 ml of 0.1% alcoholic solution of ortho-cresolphthalein with 0.05*N* alcoholic KOH to a definite pink color. Twenty ml of boiling ethanol was then added, and the titration was carried again to the definite pink color. The increase in titration of the sample during the 24 hr incubation period represents the measure of proteolysis.

Extract Preparation. In extracting the proteolytic enzymes from the samples, several extraction media were used; namely, water, 5% sodium chloride solution, 30% ethanol, and 30% and 50% glycerol. To 20-g samples of the ground defatted beans, 80 ml of each of the respective extraction fluids were added. The extraction was carried on with occasional shaking for 24 hr at 30°C, using toluene as an antiseptic. At the end of this period the mixture was centrifuged, and the clear extract subjected to the test method. Extraction of the proteolytic enzymes with water proved to be inefficient, giving titration values of only approximately 0.1 ml of 0.05*N* alkali solution. Extraction with 30% and 50% glycerol yielded the most active extracts. The values reported in Table III and Figure 2 were obtained with 50% glycerol extracts.

Nature of Soybean Protease. No attempt was made in these studies to isolate the various components that are no doubt present in the

TABLE III

EFFECT OF GERMINATION ON SOYBEAN PROTEASE

(pH 7.0—Citrate buffer)

Experiment No.	Type of material	Proteolytic activity expressed in ml *N*/20 KOH per 10 g		
		Soybean variety		
		Y	I	C
1	Soybean, original	1.63	1.37	1.21
2	Soybean, germinated 6 days	4.03	2.95	2.43
3	Soybean, germinated 12 days	6.86	5.85	5.39

soybean protease system. In this discussion, the term "protease activity" includes depeptidases, and polypeptidases, as well as proteinases. For this system, we propose the name "Soyin."

Discussion of Results. Experiments recorded in Table III show that during germination there is a very considerable increase in proteolytic activity. Three types of soybean, "Y," "I," and "C," indicate some variation between soybean varieties, but the increase is comparable in all cases.

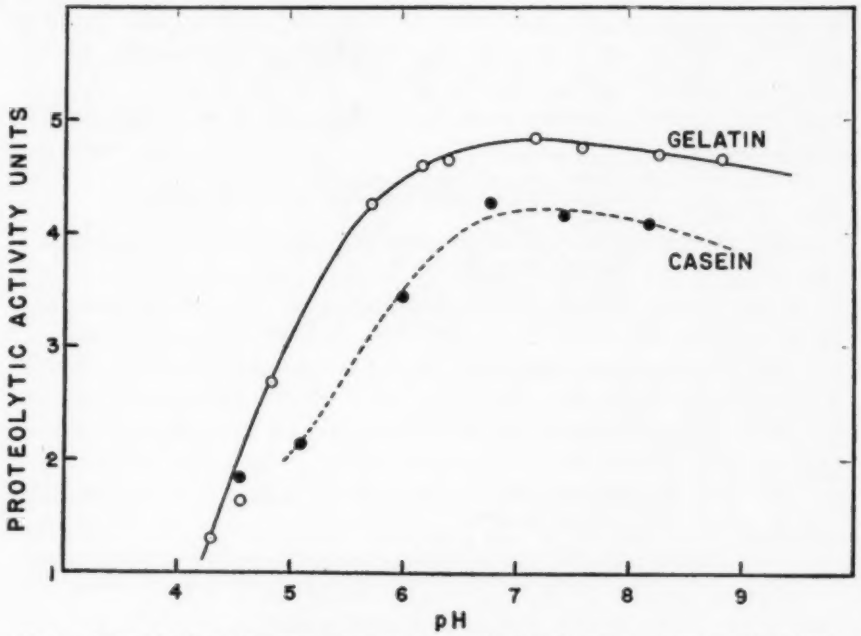

Fig. 2. Effect of pH on the proteolytic activity of a 50% glycerol extract of germinated soybeans. Proteolytic activity units; ml of 0.05*N* KOH per 10 g soybeans.

In Figure 2 is recorded the soybean proteolytic activity with varying pH values, controlled by adding varying amounts of citrate buffer to the digests, using the substrate gelatin in one set of experiments and casein in the other. With increasing pH, the activity increased rapidly until an optimum was reached between pH 6.5 and pH 7.0. A further increase in pH caused only a slight decrease in activity.

Summary

Soybean seeds are a good source of beta-amylase. They contain, however, only a trace of alpha-amylase. Germination does not affect these enzymes to any extent. Unlike barley and wheat, soybeans do not contain bound beta-amylase. The optimum pH of the soybean amylase has been found to be in the neighborhood of pH 5.9.

"Soyin," the protease of soybean, increases considerably during germination. It is extracted efficiently with 30% and 50% glycerol solutions. On casein and gelatin substrates, the activity of "Soyin" increases rapidly with increasing pH, until an optimum is reached at approximately pH 6.7–7.0, and decreases slightly in the alkaline range.

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THE LOSS OF THIAMINE ON COOKING BREAKFAST CEREALS

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The stability of thiamine in natural substances has long been known to depend on temperature (Holst, 1907; Chick and Hume, 1917; Vedder, 1918; Sherman and Grose, 1923; and Keenan, Kline, Elvehjem, and Hart, 1935). That pH also influenced the destruction of the vitamin by heat was demonstrated by Sherman and Burton (1926). Considerable data have been accumulated regarding the effect of these

factors on the loss of thiamine during the preparation of foodstuffs under various conditions.

Destruction of pure thiamine on heating depends on time and pH (Farrer, 1941). Beadle, Greenwood, and Kraybill (1943) studied the effect of temperature and pH on solutions of thiamine and found that the amount of destruction depended not only on time, temperature, and pH, but also on the nature of the electrolyte present. Cocarboxylase, the pyrophosphate of thiamine, was more stable than the vitamin under similar test conditions (Greenwood, Beadle, and Kraybill, 1943).

The experiments reported herein were carried out to investigate the relative stability of thiamine and cocarboxylase, and to determine the cooking loss of the vitamin in cereals fortified with thiamine and cocarboxylase.

Experimental

The thiochrome method of Hennessy and Cerecedo (1939), as modified by the Research Corporation Committee on the Thiochrome Method (Hennessy, 1941), was used throughout this investigation.

Destruction of Thiamine and Cocarboxylase in Buffered Solutions. Five μg of synthetic thiamine hydrochloride and 5-μg equivalents of thiamine as cocarboxylase were placed in separate 25 ml volumetric flasks containing 10 ml of a standard buffer solution. McIlvaine's citric acid-disodium phosphate buffer was used to acquire pH values up to 6.25 and Clark and Lubs' potassium acid phosphate-sodium hydroxide buffer was employed for pH values greater than 6.25. The flasks were autoclaved for 15 min at 120°C, cooled immediately, and neutralized to pH 4.5, using bromocresol green as an outside indicator. One ml of a 10% takadiastase solution was then added to each flask and the volume adjusted to 25 ml. The contents of the flasks were mixed and incubated overnight at 40°C. The same procedure was carried out on control solutions except that autoclaving was omitted.

The results illustrated in Figure 1 demonstrate that cocarboxylase was more labile to heat than thiamine at each pH tested. This cannot be attributed to incomplete splitting of the cocarboxylase by the enzyme, since unheated controls showed practically quantitative recovery.

The Effect of pH on the Loss of Thiamine and Cocarboxylase During Cooking. To show a practical application of the above results, two samples of farina were studied. One was fortified with thiamine and the other with cocarboxylase (approximately 4-μg equivalents of thiamine per g). Five-g samples of each farina were weighed into 100 ml Kohlrausch flasks and 40 ml of distilled water added. Five

different pH values were obtained by adding disodium phosphate at levels of 0.0, 0.5, 1.0, and 4.0 percent. The samples were placed in a flowing steam cooker (100–101°C) for time intervals of 5, 15, 30, and

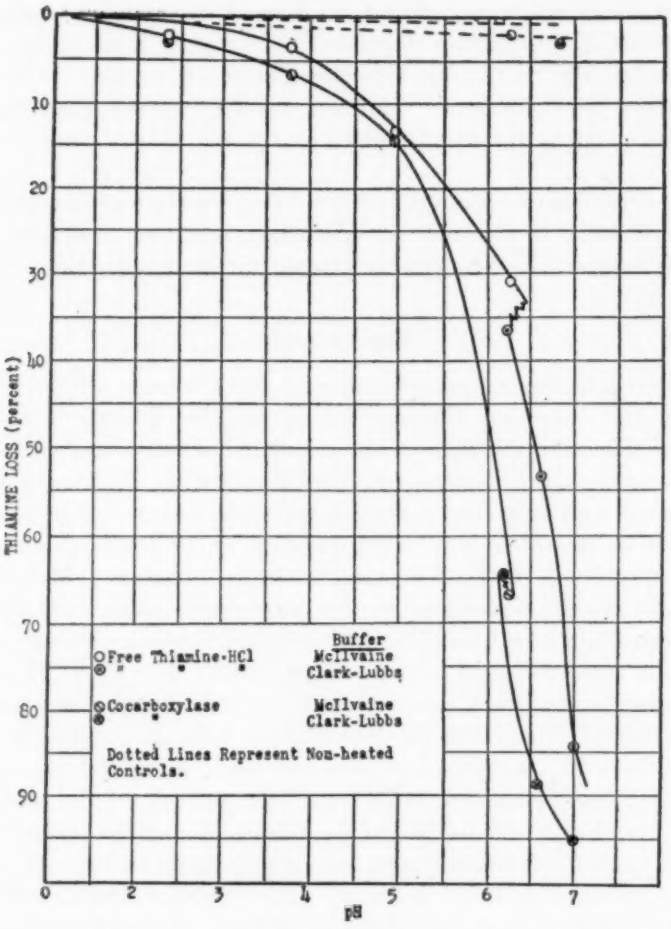

Fig. 1. Comparative stability of cocarboxylase and thiamine hydrochloride to heat at different hydrogen-ion concentrations. Solutions containing 0.5 μg equivalents of thiamine hydrochloride were autoclaved for 15 min at 15 lb pressure.

60 min. Control samples were treated with 5 ml of 1N H_2SO_4 before cooking.

After cooking, 5 ml of 1N H_2SO_4 were added to all of the samples except the controls, and the entire series autoclaved at 120°C for 15 min. The samples were cooled and incubated overnight at 40°C with takadiastase. The solutions were neutral to bromocresol green (pH

4.5) before incubation. A Coleman electrometer was used to determine the pH of the cooked samples.

Tables I and II show that the cooking loss increased with pH and time of cooking, and was greater for farina enriched with cocarboxylase than for that enriched with pure thiamine.

TABLE I

LOSS OF THIAMINE DURING COOKING OF FARINA FORTIFIED WITH PURE THIAMINE HYDROCHLORIDE (100–101°C)

Sample	pH after cooking	Cooking time in minutes							
		5		15		30		60	
		Thiamine	Loss	Thiamine	Loss	Thiamine	Loss	Thiamine	Loss
		μg/g	%	μg/g	%	μg/g	%	μg/g	%
Control	2.0	4.14		4.14		4.14		4.14	
0.0% $Na_2HPO_4 12H_2O$	5.8	4.17	+0.7	4.05	2.2	3.91	5.56	3.72	10.1
0.5% $Na_2HPO_4 12H_2O$	6.6	4.35	+5.08	3.90	5.8	3.86	6.76	3.32	19.8
1.0% $Na_2HPO_4 12H_2O$	6.9	3.92	5.32	3.64	12.1	3.31	20.0	2.89	30.2
2.0% $Na_2HPO_4 12H_2O$	7.2	3.72	10.1	3.42	17.4	2.66	35.8	2.00	51.7
4.0% $Na_2HPO_4 12H_2O$	7.5	3.56	14.0	3.24	21.8	1.64	60.5	0.74	82.2

TABLE II

LOSS OF THIAMINE DURING COOKING OF FARINA FORTIFIED WITH PURE COCARBOXYLASE (100–101°C)

Sample	pH after cooking	Cooking time in minutes							
		5		15		30		60	
		Thiamine	Loss	Thiamine	Loss	Thiamine	Loss	Thiamine	Loss
		μg/g	%	μg/g	%	μg/g	%	μg/g	%
Control	2.0	3.80		3.80		3.80		3.80	
0.0% $Na_2HPO_4 12H_2O$	5.8	3.89	+2.3	3.48	8.42	3.12	17.9	2.90	23.7
0.5% $Na_2HPO_4 12H_2O$	6.6	—	—	—	—	2.77	27.1	2.01	47.1
1.0% $Na_2HPO_4 12H_2O$	6.9	—	—	2.88	24.2	2.14	43.7	1.49	60.8
2.0% $Na_2HPO_4 12H_2O$	7.2	—	—	2.49	34.5	1.57	58.7	0.84	77.9
4.0% $Na_2HPO_4 12H_2O$	7.5	—	—	1.62	57.4	0.76	80.0	0.27	92.8

Thiamine Loss During Cooking of Some Commercial Breakfast Cereals. For this study, samples of commercial cracked wheat, oatmeal, enriched farina, and a quick-cooking farina (with increased pH) were employed. The thiamine content of the samples was determined in the usual manner. The samples were then cooked for intervals of 5, 15, 30, 60, and 120 min at 100–101°C in a flowing-steam cooker. After cooking, determinations of pH and of thiamine content were made.

Table III shows that, in general, the vitamin loss during cooking increased with increasing pH values. None of the cereals sustained a significant loss of thiamine for cooking periods of 5 and 15 min. The

TABLE III

LOSS OF THIAMINE DURING COOKING OF BREAKFAST CEREALS (100–101°C)

Cereal	Thiamine content	pH after cooking	Cooking time in minutes									
			5		15		30		60		120	
			Thiamine	Loss	Thiamine	Loss	Thiamine	Loss	Thiamine	Loss	Thiamine	Loss
	μg/g		μg/g	%	μg/g	%	μg/g	%	μg/g	%	μg/g	%
Quick-cooking farina, enriched	5.42	6.85	5.43	0.0	4.91	9.4	4.46	17.7	4.07	25.0	3.21	40.8
Cracked wheat	4.47	6.30	4.28	4.25	3.89	13.0	3.88	13.2	3.61	19.2	3.28	26.6
Oatmeal	5.74	6.20	5.75	0.0	5.60	2.44	5.38	6.28	5.18	9.77	4.60	19.9
Enriched farina	4.14	5.80	4.17	0.0	4.05	2.2	3.91	5.56	3.72	10.1	3.52	15.0

cracked wheat and quick-cooking farina both showed appreciable losses during the longer cooking times.

Discussion

Some early investigators reported that heating or cooking of foodstuffs did not destroy thiamine, while other workers presented evidence to show that destruction did occur. Williams (1939) suggested that much of the conflicting evidence was due to differences in the thermolability of the free vitamin and cocarboxylase. The results of the present study indicate that foodstuffs rich in cocarboxylase would show more cooking loss than those rich in the free vitamin. Our data are not in accord with the report by Greenwood, Beadle, and Kraybill (1943) that cocarboxylase was more stable to heat than the free vitamin. However, the test conditions in their experiments were different from those described here; since these workers observed that the stability of pure thiamine at any one pH depended on the buffer system employed, it is possible that cocarboxylase may manifest similar properties.

Although 5 min is sufficient time to cook breakfast cereals such as farina and oatmeal, the time is often extended in many cases. This is especially true in the preparation of cooked cereal for large groups, such as in our armed forces, or in restaurants and institutions where it may be left on a steam table for several hours. Prolonged cooking is undesirable since there is a significant loss of thiamine, especially in cereals with a high pH.

Summary

Pure cocarboxylase was more readily destroyed by heat than thiamine in buffered solutions at pH values of 3.5 to 7.0.

The vitamin loss during cooking, at pH values between 5.8 and 7.5, was greater in the case of farina fortified with cocarboxylase than when thiamine was used.

Cooking tests with enriched farina, oatmeal, cracked wheat, and quick-cooking enriched farina showed that the thiamine loss was negligible for short cooking periods. The loss was increased when the cooking time was prolonged, especially in the instance of cracked wheat and farina which were cooked at pH levels of 6.3 and 6.8 respectively.

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THIAMINE RETENTION IN THE COMMERCIAL PRODUCTION OF ZWIEBACK

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There is considerable interest in the extent of loss of thiamine in bakery products during processing. The Baking Industry Advisory Committee of the War Food Administration has recommended the extension of the enrichment program to baked goods other than bread which are capable of conveying to the consumer a full measure of the vitamins added. Factual data on the bake-out loss of thiamine in the production of many of the sweet, yeast-raised products are lacking. The present report provides some data on the retention of thiamine in the commercial preparation of Zwieback, a product which is subjected to both baking and toasting operations.

Materials and Methods

A commercial dough of the customary size was enriched by the use of tablets [1] in a commercial bakery. The dough formula which was selected and other pertinent information are provided in Table I. The

TABLE I

DOUGH FORMULA SELECTED FOR ZWIEBACK

Ingredient	%	lb	oz
Flour	100	300	—
Water	53	159	—
Yeast	2	6	—
Salt	0.8	2	4
Sugar	14	42	—
Dried skim milk	1	3	—
Malt	2	6	—
Shortening	4	12	—
Flavoring materials	0.11	—	5.5
Vitamins and iron	tablets	—	—

Mixing time—7 min (high speed).
Dough temperature out of mixer—26.1°C (79°F).
Total fermentation time—3 hr at 26.7°C (80°F); (punched at 2½ hours).

dough was divided into 14-oz (400 g) loaves by a Baker Perkins six-pocket divider.

Considerable pains were taken to secure representative samples for thiamine assay of the dough, of the bread made therefrom, and of the

[1] The product known as B-E-T-S of the Winthrop Chemical Co. was used.

finished Zwieback. Four loaves, one from each quarter of the dough, were accurately weighed (400 g) and panned. These loaves were removed from the pan for assay after proofing, as the rest of the dough went to the oven. The loaves were flattened upon one another and a cross section cut for assay.

In a like manner, 16 other loaves, one loaf taken from each third cut as the dough passed through the divider, were weighed, panned in four four-strap pans, and proofed. A Baker Perkins tunnel type oven which was used for baking and toasting accommodates five of these pans from side to side of the oven. To obtain loaves baked at both center and side of the oven, the 12 loaves contained in the three pans from the center to one side of the oven were selected for slicing and toasting. The remaining pan, on the opposite side between center and side pan, was used for the assay of the untoasted loaves. The oven temperatures and time of baking and toasting are given in Table II.

TABLE II

OVEN TEMPERATURE AND TIME IN PRODUCTION OF ZWIEBACK
(Tunnel type oven)

Operation	Temperature								Total time in oven
	First section		Second section		Third section		Fourth section		
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom	
	°C	°C	°C	°C	°C	°C	°C	°C	*min*
Baking	168	204	141	174	177	154	160	168	27
Toasting	174	188	132	146	177	152	154	166	19

After cooling and drying about 24 hours on the rack, the 12 loaves were put through the slicing machine and cut into $\frac{5}{8}$-inch slices. Weighings were made before and after slicing to record the weight of crumbs lost in slicing. The slices were laid as close together as possible on rectangular heavy metal screens for toasting; five screens just filled the space from side to side on the floor of the oven. The 12 loaves covered more than three screens, giving a representative sample from side to center of the oven. All screens were covered with slices and the oven was full throughout the toasting.

The Zwieback obtained from the entire 12 loaves was ground and thoroughly mixed for a sample. Likewise the four loaves set aside for bread analysis were sliced, air-dried, ground, and thoroughly mixed.

Thiamine was determined by the collaborative thiochrome method of Hennessy (1942), as outlined in Cereal Chem. Bull. 2 (**2**): 25–29, and

the results expressed in terms of the total thiamine present in four loaves in the form of dough, bread, and Zwieback respectively.

Results and Discussion

It will be noted from the results in Table III that the percentage of the total thiamine of the dough lost in toasting the sliced loaves (20%),

TABLE III

MEAN THIAMINE LOSSES IN BAKING AND TOASTING

Milligrams of thiamine in four loaves			Percent of total thiamine of dough lost		
Dough	Bread	Zwieback	In baking	In toasting	Total loss
4.86	3.99	3.02	18	20	38

is not much greater than the percentage lost in baking the loaves (18%). This is probably due to the comparatively long, flat shape of the loaves, which results in a low ratio of crumb to outer surface of loaf. Schultz, Atkin, and Frey (1942) have shown that thiamine destruction in baking is much greater in the crust than in the crumb of the loaf of bread.

If the toasting loss is calculated in terms of the percentage of the total thiamine of the bread instead of the dough, we find the toasting loss to be 24%. The relatively high sugar content of this product results in obtaining the desired color without the application of unduly high temperatures or long toasting times, either of which would result in greater destruction of thiamine. Hoffman, Schweitzer, and Dalby (1940) found a loss of 26% in the preparation of Melba toast from high B_1 white bread. Melnick, Mabardie, Bernstein, and Oser (1941) reported a 15% destruction of thiamine in preparing "usual toast." Downs and Meckel (1943) found a toasting loss of 13% of the total thiamine of enriched bread in 50 sec toasting at the temperature of the household toaster. This was the degree of toasting preferred by a majority of persons asked to view the toast samples.

Summary

A representative Zwieback dough was enriched with commercial enrichment tablets, and the dough, bread, and toast were assayed for thiamine by the thiochrome method. The over-all loss of thiamine was 38%, attributable almost equally to the baking and toasting operations, under the conditions of the experiment.

The toasting loss was 24% when calculated on the total thiamine of the bread.

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A PREHARVEST SURVEY OF THE PROTEIN CONTENT OF WESTERN CANADIAN WHEAT OF THE 1943 CROP[1]

J. ANSEL ANDERSON,[2] K. W. NEATBY,[3] and I. LEVI[4]

(Manuscript received, April 13, 1944; presented at the Annual Meeting, May 1944)

Since 1927 the Board of Grain Commissioners' Laboratory has made annual surveys of the protein content of Western Canadian hard red spring wheat, and data for the first 12 of these have been summarized by Anderson and Eva (1943). Because the annual survey cannot be completed until samples have been obtained from northern areas where the harvest is late, and because additional time is required for mapping, tabulating, typing, and printing, it has rarely been possible to distribute the survey report before mid-November. Some information has been made available earlier by issuing preliminary and interim reports to the press, but these cannot contain detailed data. In one year when conditions were advantageous the final report was distributed on October 29, but in some years it has been delayed until December. Moreover, for the past three years, transport and storage problems have made it necessary to place certain restrictions on the delivery of wheat, especially during the early part of the crop year, and this has made it increasingly difficult to obtain samples for an early protein survey. In these circumstances it seemed desirable to investigate the possibilities of a preharvest survey. An initial study was made in 1943 and is reported in this paper.

Other similar studies have dealt with preharvest surveys of yield, and King and Jebe (1940) have reviewed the literature and contributed a valuable study of sampling problems. In their survey, a two-man team covered each crop-reporting district by car, following three evenly

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spaced routes running north and south and three running east and west. One field was sampled after every two miles of wheat frontage, and a procedure for ensuring random sampling within fields was followed. The investigation showed that this method of estimating and forecasting yield per acre was both practical and efficient.

For our study, a systematic attempt to approach the ideal of random sampling did not seem feasible. The area to be covered was too large, and with current shortages of manpower, gasoline, and tires, the use of a car was impossible. The investigation was therefore designed to determine whether the simplest practical method of making a preharvest survey would give results comparable with those obtained in the postharvest survey. For the postharvest surveys, it is the practice to collect 5,000 or 6,000 samples of farmers' deliveries to elevators in such a way that numbers of samples are roughly proportional to production in each district. But for the preharvest survey, only about 1,500 samples—taken by country elevator agents from wheat fields just prior to harvesting—were collected, and no attempt was made to obtain proportionality between sampling and production. In both surveys, only a general picture of the distribution of zones of different protein levels was sought; indeed, this is all that is required for practical purposes. For this reason it was not necessary to design the investigation with detailed statistical analyses of the data in mind. It was thought that maps would serve best to summarize and present the results in such a way that the reader could check the writers' conclusions. A liberal use of maps has therefore been made.

Materials and Methods

Preharvest Samples. The samples for the preharvest survey were collected by asking 3,316 of the agents at country elevators in Western Canada to collect one sample apiece in accordance with the instructions given below. It will be apparent that the task of taking samples was made as simple as possible; a deliberate attempt was thus made to facilitate the cooperation of busy agents.

Who Takes Samples? Each buyer of a Line Elevator Company is asked to obtain one sample.

Selection of Wheat Field. If you are the only Line Elevator buyer at point you may take the sample from any conveniently located field of wheat. If there are two or more Line Elevator buyers at your point, it will be necessary for you to get together and arrange to take samples from different farms.

Time for Taking Sample. The sample shall be taken as soon as the field is ripe and before it is cut.

Method of Taking Sample. It is left to your good judgment to take an average sample of the field. Avoid the early spots on high ground, and the late spots in hollows.

Size of Sample. The investigation requires a sample of about 4 ounces (130 grams). Collect enough heads to yield this amount.

Threshing of Sample. If the sample is not dry enough for immediate hand threshing, hang it up in your office for a day or two. When the heads are dry, put them in a small cloth bag and beat them with a stick. Empty the bag, blow off the chaff, and enclose the grain in a sample envelope on which appears your company name and shipping point.

Mailing of Sample. Stick the enclosed label to sample envelope, and mail.

A total of 1,620 samples was received (49% of requests), but 116 of these were spoiled because they were not dried sufficiently before shipment; 1,504 samples were therefore available for analysis.

Postharvest Samples. In the early part of the crop year it is the practice for elevator agents at all points to send many envelope samples of farmers' deliveries of new wheat to the head office of their companies. This procedure makes it possible to check the uniformity of buying operations at all points. Through the kindness of the companies these samples are made available to the Grain Research Laboratory. From them there is selected a series of samples of the contract grades which provides good coverage of all wheat-growing areas. By making the number of samples taken from each crop district proportional to wheat production in the district, the survey is "weighted" to give a close estimate of the mean protein content of the crop. This method is the best of the procedures that have been used by this laboratory. For further information on methods, the reader is referred to the bulletin by Anderson and Eva (1943) dealing with protein surveys of Western Canadian wheat for the 12 years 1927 to 1938.

Analytical Methods. It was necessary to air-dry some of the preharvest samples in the laboratory. All samples were cleaned in an Emerson Kicker, and moisture was determined with a motor-driven Tag-Heppenstall moisture meter calibrated to give the same results as the Brown-Duvel method. Nitrogen determinations were made by the Kjeldahl-Gunning-Arnold method on a 1-g sample of ground wheat (Hobart coffee mill) and protein data (N × 5.7) are reported on a 13.5% moisture basis.

Preparation of Maps. Two types of maps, dot and hatched, are presented. Separate dot maps are used for different protein ranges, and one dot is shown for each shipping point having a mean protein level within the specified range. Hatched maps are also based on the mean protein levels for shipping points. The mean data are transferred to a 12 × 20 inch base map on which a different colored dot is used for each 1.0% range in protein content. As the mean protein levels tend to change relatively uniformly from one district to another, it is not difficult to draw zone boundaries separating dots of different colors. When one or two shipping points have protein levels outside the range for surrounding points, the area is frequently too small to show as a separate zone; but when two or three such points are fairly

close together it is generally possible to outline a zone that is large enough to reproduce on a small-scale map. Zone boundaries are subsequently transferred to a 6 × 10 inch base map, the zones are hatched with "Zip-A-Tone," and the map is photographed.

Results and Discussion

It is important to deal with the relative dates by which pre- and postharvest surveys were completed, and this point is discussed first. The accuracy with which the preharvest survey predicted the geographic distribution of zones of different protein level—as determined in the postharvest survey—is then considered with the aid of dot and zone maps for both surveys. And in order to provide perspective, the preharvest zone map is also compared with zone maps for each of the past 16 years. Mean protein levels for crop districts, correlation and regression coefficients for pre- and postharvest means, and the accuracy with which the mean protein levels for the whole crop were predicted, are then discussed.

Comparative Rates of Collection of Samples. The rates at which samples were received are illustrated in Figure 1. By October 1,

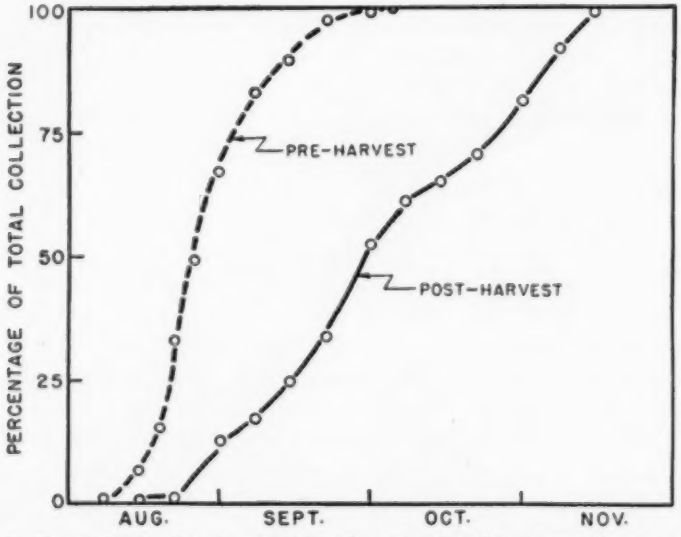

Fig. 1. Curves showing the rates at which preharvest and postharvest samples were received.

almost all of the 1,620 samples for the preharvest survey had been received, but only 52% of the 5,645 postharvest samples had been collected. In both surveys the principal difficulty is that of obtaining samples from northern areas where the harvest is late. For instance, almost all of the preharvest samples received after September 15 came

from north-central Alberta and the Peace River Block. Thus in 1943 it would have been impossible to cover the whole crop-growing area adequately before October 1 unless the northern samples had been taken before the grain was ripe. In spite of this limitation, the preharvest survey was completed over a month before the postharvest survey. This represents an appreciable saving of time; and in many years, when the harvest is reasonably early, it should be possible to complete the preharvest survey two weeks earlier. When the harvest is later in the North, it may well prove practical to take the samples on September 10, and to make special arrangements for drying and shipping them, so that the survey can be completed by mid-September.

Preliminary maps covering southern and eastern areas where the harvest is early can be prepared several weeks earlier for both surveys. This practice has been followed for some years with the postharvest surveys. In the present study, however, the object was to compare procedures for obtaining reasonably accurate maps for the whole of the crop-growing area. Accordingly, it does not seem advisable to discuss preliminary maps.

Dot Maps for Pre- and Postharvest Surveys. A pair of maps representing pre- and postharvest data are shown in Figure 2 for each of four protein ranges: under 11.0%, 11.0 to 12.9%, 13.0 to 14.9%, and over 14.9%. In each map, one dot was entered to represent each shipping point that had a mean protein level within the range for the map. The number of shipping points represented on each map, and the number of points common to both maps, are shown in Table I.

TABLE I

NUMBER OF SHIPPING POINTS REPRESENTED IN EACH SURVEY

Protein range for maps	Number of shipping points		
	Preharvest map	Postharvest map	Common to both maps
Under 11.0%	63	49	9
11.0 to 12.9%	247	392	102
13.0 to 14.9%	494	836	282
Over 14.9%	224	124	50
Total	1,028	1,401	

It is not entirely fortuitous—though unpremeditated—that so few of the shipping points are common to both surveys; four large companies which supplied the majority of the postharvest samples were not asked to collect preharvest samples. This disparity between surveys creates no special difficulty in interpreting the data by means of maps,

but may have caused slightly greater variation in the mean values for crop districts (discussed in a later section) than would have occurred if the same set of points were represented in both surveys.

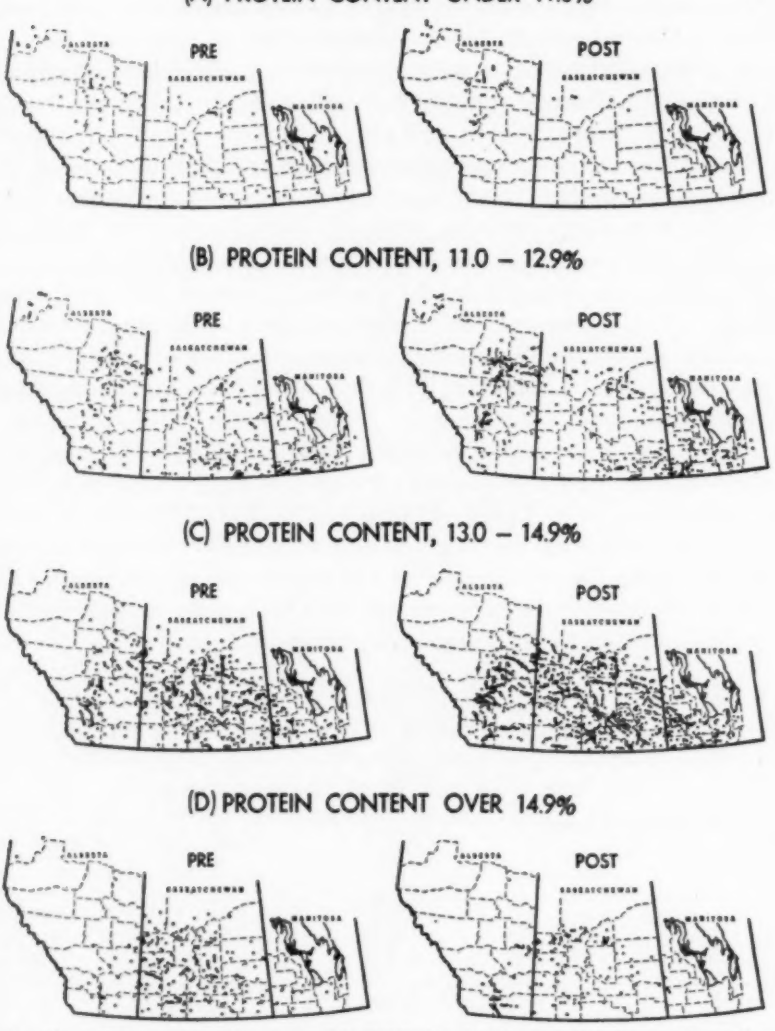

Fig. 2. Maps of Western Canada showing the distribution of shipping points from which preharvest and postharvest samples of different protein ranges were received.

The dot maps represent the most extensive and detailed treatment of the raw data presented in this paper. Nevertheless they can be allowed to speak for themselves. The reader will doubtless agree that there is great similarity between the geographic distributions of dots

within each pair of maps. But when the distribution of dots between preharvest maps is compared with the distribution between postharvest maps, considerable differences are apparent. These result largely because the number of samples taken from different areas was made roughly proportional to production in the postharvest survey, whereas it was not consistent with the required simplicity to "weight" the preharvest collections in this manner; accordingly, the areas of heavy production, in which protein levels of 13.0 to 14.9% predominate, are

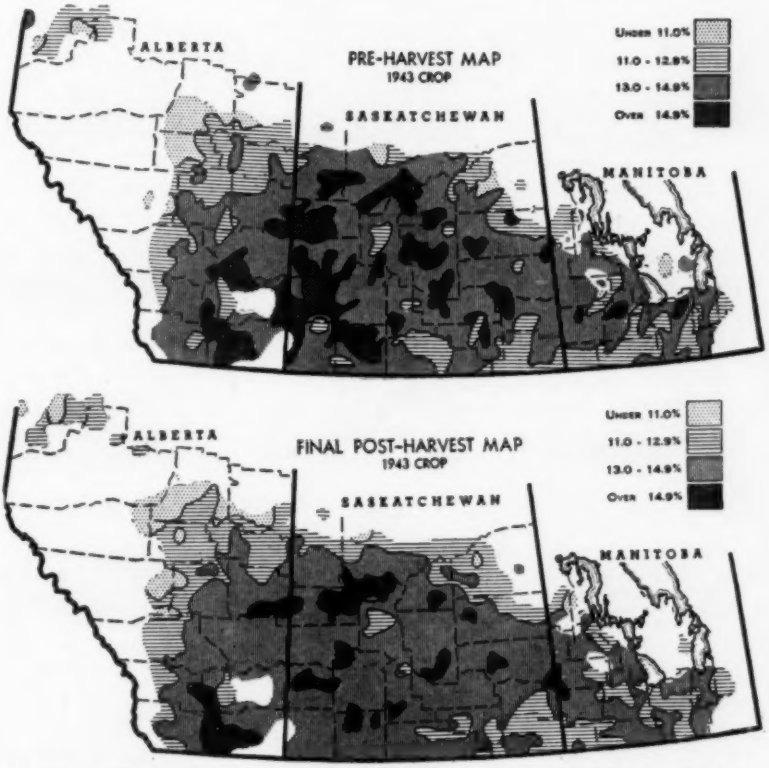

Fig. 3. Maps of Western Canada showing protein zones outlined from the data for the preharvest survey (top), and from the data for the postharvest survey (bottom).

better represented in the postharvest survey. However, this difference in method does not seem adequate to explain why the preharvest map for the highest protein range shows considerably more shipping points in central and southern Saskatchewan, and in southeastern Alberta, than does the postharvest map. It seems probable that the preharvest survey overestimated protein levels in parts of these areas. The writers have not been able to find an adequate and convincing explanation of this difference between the two surveys. It may well be related

to variations in the weather at harvest times in different districts; but the area covered by the surveys is so large, and the detailed information on weather is so meager, that investigation of this matter does not appear feasible.

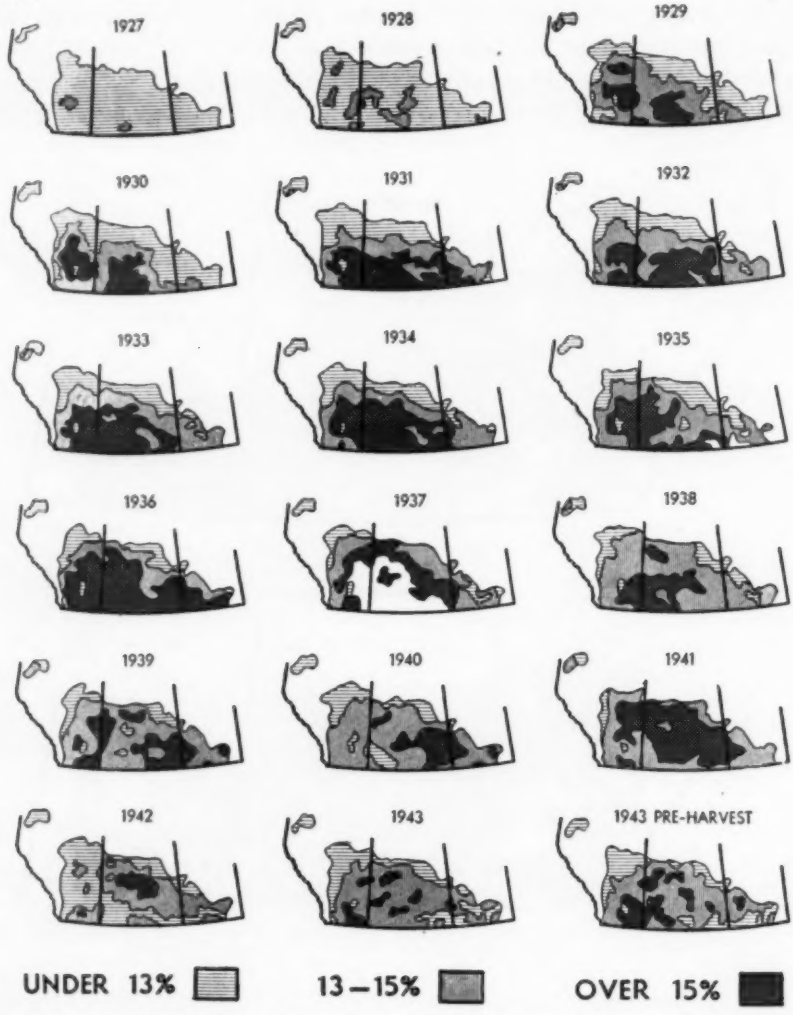

Fig. 4. Protein maps of Western Canada for the postharvest surveys of the years 1927 to 1943, and for the preharvest survey of 1943.

Hatched Maps for Pre- and Postharvest Surveys. The data presented in Figure 2 are summarized in the pair of hatched maps that appear in Figure 3. The preharvest map (top) shows larger zones of high protein level (dark crosshatching) than the postharvest map (bottom), especially throughout eastern Saskatchewan. Though the agreement for

other zones is by no means perfect, the writers believe that it is adequate for practical purposes and better than might have been expected in view of the methods used for the two surveys.

Comparison of 1943 and Earlier Maps. The usefulness of the preharvest map for 1943 can be determined—and put in perspective—by comparing it not only with the postharvest map for 1943 but also with those for as many other years as possible. Accordingly, maps for the years 1927 to 1942, and the two maps for 1943, are shown in Figure 4. As these maps are reproduced on a small scale, only one range is shown below 13.0% protein, and the zones are outlined in less detail; some of the smaller ones are left out, and others have been joined to make a single, larger area.

It will be readily agreed that the preharvest map for 1943 resembles the postharvest map for 1943 much more closely than it resembles the postharvest map for any of the other 16 years. The writers believe that this in itself is enough to establish the preharvest survey—carried out by the simple means used in this investigation—as a promising procedure for obtaining early information on each crop.

Mean Protein Levels. In order to make a reasonable comparison of the pre- and postharvest data in terms of mean protein levels, it is necessary to discount the normal variation—frequently in units of as much as 5%—that occurs between samples collected from one shipping point. This can be done by working with areas that are large enough to be represented by a considerable number of samples; and for this purpose the crop districts (Fig. 5) are a logical choice.

Fig. 5. Map of Western Canada showing crop districts and principal towns.

The mean protein levels and the number of samples for each crop district, for both pre- and postharvest surveys, together with data on wheat production, are given in Table II. Agreement between the pre-

TABLE II

PROTEIN LEVELS, NUMBERS OF SAMPLES, AND PRODUCTION FOR CROP DISTRICTS

Province	Crop district	Protein content		Number of samples		Production
		Pre-harvest	Post-harvest	Pre-harvest	Post-harvest	
		%	%			*1,000 bu.*
Saskatchewan	4B	15.2	14.6	52	76	2963
Alberta	1	15.0	15.0	23	125	5239
Saskatchewan	7B	15.0	14.7	40	112	3661
Saskatchewan	3BS	15.0	14.2	60	140	9414
Saskatchewan	8B	14.5	14.2	49	214	8314
Saskatchewan	1B	14.5	13.4	17	119	6765
Saskatchewan	7A	14.4	14.3	68	145	5477
Saskatchewan	3BN	14.4	14.1	70	152	7834
Saskatchewan	6A	14.4	14.1	74	177	10680
Saskatchewan	2B	14.2	14.1	66	209	13797
Alberta	3	14.2	13.9	20	76	3451
Alberta	5	14.1	14.0	22	122	3655
Saskatchewan	5B	14.1	13.7	64	203	8837
Saskatchewan	3AN	14.0	13.8	35	129	5573
Saskatchewan	9B	14.0	13.5	26	143	6164
Saskatchewan	9A	13.9	13.9	41	164	9530
Manitoba	7	13.9	13.4	22	112	4627
Saskatchewan	5A	13.8	13.8	57	185	13073
Saskatchewan	6B	13.8	13.8	63	212	10543
Alberta	4	13.8	13.7	59	194	7716
Saskatchewan	2A	13.8	13.4	34	107	7979
Manitoba	9	13.7	13.3	3	38	1756
Alberta	7	13.5	13.7	31	162	8536
Manitoba	11	13.5	13.1	4	50	2364
Saskatchewan	4A	13.4	13.9	23	43	2265
Alberta	2	13.4	13.3	30	114	4513
Manitoba	10	13.4	13.0	16	95	4576
Saskatchewan	3AS	13.3	13.2	53	206	15438
Alberta	6	13.2	13.3	46	274	13668
Manitoba	3	13.2	13.1	39	177	8793
Saskatchewan	1A	13.2	12.7	28	149	8557
Manitoba	8	13.1	13.2	22	72	3315
Manitoba	2	12.8	13.1	22	140	6835
Saskatchewan	8A	12.6	12.6	41	112	5219
Alberta	8 and 9[1]	12.4	12.6	33	182	8356
Manitoba	4, 5, and 6[1]	12.3	12.6	9	36	2177
Alberta	10	12.3	12.6	47	256	11224
Manitoba	1	12.1	12.9	19	74	3931
Manitoba	12, 13, and 14[1]	12.1	12.8	8	35	2018
Alberta	15 and 16[1]	12.0	11.6	25	77	3901
Alberta	13	11.6	11.5	6	45	2600
Alberta	11	11.1	12.0	11	83	3277
Alberta	14	10.8	11.0	17	103	7554
Alberta	12	—	10.2	—	6	456

[1] Crop districts in which little wheat was grown, and from which few samples were obtained, were grouped with adjacent districts.

and postharvest means is reasonably good: 60% of the crop districts, including most of those in which production was high, yield means differing by 0.3% or less; and for an additional 26%, the difference is not over 0.5%.

It is also interesting to compare the pre- and postharvest means for the provinces and for Western Canada as a whole. This can be done in three ways: by comparing the unweighted means (of the crop district means); by comparing means weighted in accordance with the number of samples for each crop district (actually the means for all samples); and by comparing means weighted for production in each crop district. The three sets of data are shown in Table III.

TABLE III

MEAN PROTEIN LEVELS FOR CROP DISTRICTS FOR EACH SURVEY

Province	Survey	Mean protein levels		
		Of crop district means	Weighted by number of samples	Weighted by production
Manitoba	Preharvest	13.0	13.0	13.1
	Postharvest	13.0	13.1	13.1
Saskatchewan	Preharvest	14.1	14.1	14.0
	Postharvest	13.8	13.8	13.8
Alberta	Preharvest	12.9	13.1	12.9
	Postharvest	12.9	13.1	13.0
Western Canada	Preharvest	13.46	13.75	13.55
	Postharvest	13.36	13.47	13.43

The three methods of calculating the means give much the same results. All three methods show good agreement for Alberta and Manitoba between pre- and postharvest means, but it is apparent that the preharvest survey overestimated the protein level for Saskatchewan. The agreement for Western Canada as a whole is very good; the means weighted by production, the best method of obtaining an accurate estimate, show a difference of only 0.12% between pre- and postharvest values. As there may well be some doubt as to whether the postharvest survey is accurate to $\pm 0.1\%$, or even to $\pm 0.3\%$, the estimate provided by the preharvest survey must be considered eminently satisfactory.

Correlation Studies. The correlation between the pre- and postharvest protein levels for crop district means is $r = 0.93$ ($n = 41$). The scatter diagram is shown in Figure 6. Different types of dots have been used for the data for each province, and it is apparent that the swarms are relatively homogeneous.

The regression coefficient is 0.75. Under ideal conditions, and with a correlation of 1.0, a regression coefficient of 1.0 might also be obtained. As it is a property of the regression coefficient to decrease when the correlation coefficient falls as a result of extraneous random variations,

the writers are not inclined to stress the fact that the regression coefficient is appreciably lower than 1.0. Although the regression line suggests that the preharvest survey tends to underestimate the low protein values and to overestimate the high protein values, it appears wise to hold this hypothesis in abeyance pending the results of additional studies.

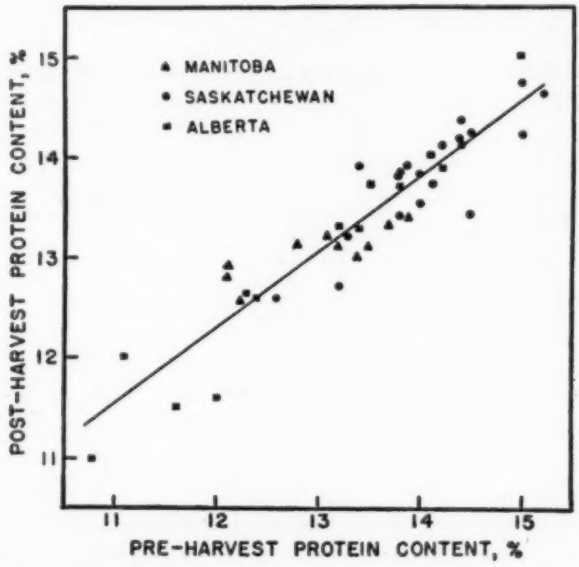

Fig. 6. Scatter diagrams illustrating the correlation between preharvest and postharvest data for the mean protein levels of crop districts in Western Canada.

As a matter of interest, the equation for predicting postharvest values from preharvest data was calculated. It is

$$y = 0.754x + 3.2 \qquad (\pm 0.3)$$

where y is the postharvest protein level and x is the preharvest value. The standard error of estimate for this equation is ± 0.3; and this appears to be a very useful level of precision.

Summary

A preharvest survey of the protein content of the 1943 crop of Western Canadian wheat was made with 1,504 samples collected just before harvest by country elevator agents. The results were compared with those given by a postharvest survey made with 5,645 samples taken from grain delivered by farmers to country elevators.

The preharvest protein map resembled the postharvest map much more closely than it resembled corresponding postharvest maps for

any of the preceding 16 years. A correlation coefficient of 0.93 ($n = 41$) was obtained between the pre- and postharvest data for the mean protein levels of crop districts; 86% of the pre- and postharvest means agreed within 0.5%. For the protein content of the whole crop, the preharvest data gave 13.55% and the postharvest data gave 13.43%. The results of the investigations are considered eminently satisfactory, and perennial preharvest surveys are planned.

Acknowledgments

The authors are indebted to the North-West Line Elevators Association, Winnipeg, Canada, and especially to those of their elevator agents who collected samples, for their valuable cooperation in this investigation. Thanks are also given to Mr. V. G. Martin, Laboratory Assistant, Grain Research Laboratory, for preparing the figures.

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EFFECT OF BRAN ON BREAD BAKING

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(Received for publication January 28, 1944)

Recent emphasis on the production of more nutritious bread has aroused new interest in conserving for human consumption the food values of wheat. That whole wheat bread as generally produced is vastly inferior technically to white bread from the viewpoint of the consumer is a well-established fact. Reasons for this inferiority are not completely known. This study was undertaken to find some of the reasons and, if possible, ways of alleviating them in order that better bread might be produced from whole wheat or long extraction flours.

Since white flour differs largely from whole wheat in that it contains only small amounts of bran and germ, one or both of these fractions may be responsible for the undesirable characteristics of whole wheat flour. A logical approach to the problem seemed to be that of superimposing these two constituents separately upon white flour and thus studying their effects.

A review of the literature shows that a number of investigations have been undertaken concerning wheat germ (Sullivan, Near, and

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[2] Professor and Head of the Department of Agricultural Chemistry, The Ohio State University.

Foley, 1936; Sullivan, Howe, and Schmalz, 1936 and 1937; Bull, 1937; and Grewe and LeClerc, 1943) but that much less work has been reported on the effect of wheat bran. However, Willard and Swanson (1911) have recorded the influence on baking of bran and bran extracts, as well as of a number of other milling by-products. Similar results on bran extracts are related by White (1913).

Experimental

Effect of Granulation of Bran. It is a rather general opinion that bran has a mechanical effect on the process of baking, serving merely to dilute the gluten and disrupt the gluten films. If this postulation is correct, grinding the bran finer should increase the loaf volume. It was decided to investigate this property. Preliminary work in which bran was ground to different granulations in a Bantam Mikropulverizer without making any attempt to remove the adhering endosperm indicated that a maximum loaf volume would be produced at an intermemediate granulation. Since a possible explanation might be that this fine grinding injures the starch granule, as reported by Jones (1940), the following procedure was used. Three different commercial samples were freed from adhering endosperm by passing through the Mikropulverizer with screen removed, followed by sifting through a No. 40 wire on a Great Western experimental sifter. The throughs of this operation were discarded and the overs divided into five portions. These portions were sifted and reground when necessary to obtain the following samples:

1—overs 20W
2—throughs 20W, overs 40W
3—throughs 40W, overs 60 GG
4—throughs 60 GG, overs 10XX
5—throughs 10XX

Protein, ash, moisture, diastatic activity, proteolytic activity, and crude fiber were determined on these samples, as described in Cereal Laboratory Methods (4th ed., 1941). Cellulose was determined by a method similar to that of Crampton and Maynard (1938). Average diameters of the brans were determined by measuring a large number of bran particles, selected at random, under a microscope containing a scale in the eye-piece. This scale was previously calibrated with a slide micrometer.

These brans were then superimposed on three different commercial bread flours at the rate of 15% (15% moisture basis) and baked in duplicate, using a procedure described in Cereal Laboratory Methods

(4th ed., 1941) and a formula comprised of 100 g flour, 3 g yeast, 5 g sugar, 1.5 g salt, 3 g shortening, and 4 g dry milk solids. In all cases a control loaf was baked each day for comparison with the bran loaves. Since it was found that doughs containing bran were very sensitive to incorrect absorption, the combinations were baked a number of times at different levels of absorption and the maximum volume found for each combination. Results of this work are recorded in Table I. Representative photographs are shown in Figure 1.

Control 2.12 0.55 0.28 0.07

Fig. 1. Loaves of bread baked with various granulations of unwashed bran. Number under each loaf indicates average diameter of particle in mm.

In analyzing these data, the control loaf volumes were subtracted from the corresponding bran loaf volumes and 100 added to the resulting figure. These positive figures were then treated to an analysis of variance, using the second order interaction, brans × flours × granulations, as the estimate of error. The results of the variance analysis are shown at the foot of Table I.

Correlation coefficients between these figures, which express the response in loaf volume to the addition of bran, and the average diameter of the bran particles are as follows:

Flour series	Correlation coefficient
1	−0.678
2	−0.874
3	−0.844

These correlations are all significant (1% point = 0.641).

Effect of Bran Extracts. The large volume of the loaves containing finely ground bran indicates that bran must contain an ameliorating factor which increases loaf volume as well as a destructive factor which influences baking. Accordingly, water, ether, and alcoholic extracts of bran were made. The ether and alcohol were removed from their corresponding extracts by vacuum distillation and the residue added to doughs made from white flour, using the same formula as described before.

TABLE I

EFFECT OF BRAN PARTICLE SIZE ON LOAF VOLUME

Combination		Size bran particles	Absorption of bran loaf 15% m. b.	Loaf volume		
Flour	Bran			Bran	Control	Response (Diff. + 100)
		mm	%	*cc*	*cc*	*cc*
1	1–1	2.12	67.5	702	720	82
1	2–1	2.35	67.5	738	718	120
1	3–1	2.66	67.5	677	718	59
2	1–1	2.12	69.0	730	792	38
2	2–1	2.35	69.0	788	815	73
2	3–1	2.66	69.0	730	792	38
3	1–1	2.12	70.5	765	800	65
3	2–1	2.35	69.0	712	787	25
3	3–1	2.66	69.0	705	770	35
Average			68.7	727	768	59
1	1–2	1.08	67.5	737	725	112
1	2–2	0.97	69.0	720	730	90
1	3–2	1.16	68.0	725	718	107
2	1–2	1.08	69.0	785	815	70
2	2–2	0.97	69.0	832	827	105
2	3–2	1.16	69.0	770	800	70
3	1–2	1.08	70.5	808	800	108
3	2–2	0.97	69.0	770	787	83
3	3–2	1.16	69.0	747	770	77
Average			68.9	766	775	91
1	1–3	0.55	70.0	772	725	147
1	2–3	0.58	69.0	745	740	105
1	3–3	0.62	70.0	790	725	165
2	1–3	0.55	72.5	780	800	80
2	2–3	0.58	69.0	823	827	96
2	3–3	0.62	71.0	815	815	100
3	1–3	0.55	71.5	772	787	85
3	2–3	0.58	71.0	780	780	100
3	3–3	0.62	71.0	760	782	78
Average			70.6	781	776	105
1	1–4	0.28	71.0	770	720	150
1	2–4	0.33	69.0	790	730	160
1	3–4	0.30	71.0	720	710	110
2	1–4	0.28	74.0	815	815	100
2	2–4	0.33	73.0	810	800	110
2	3–4	0.30	74.5	830	815	115
3	1–4	0.28	72.5	783	780	103
3	2–4	0.33	71.5	805	787	118
3	3–4	0.30	72.0	795	800	95
Average			72.1	791	773	118

TABLE I—*Continued*

Combination		Size bran particles	Absorption of bran loaf 15% m. b.	Loaf volume		
Flour	Bran			Bran	Control	Response (Diff. + 100)
		mm	%	*cc*	*cc*	*cc*
1	1–5	0.07	70.5	802	725	177
1	2–5	0.08	71.5	750	725	125
1	3–5	0.06	71.0	743	718	125
2	1–5	0.07	73.0	833	800	133
2	2–5	0.08	74.0	845	827	118
2	3–5	0.06	73.5	805	800	105
3	1–5	0.07	72.5	818	780	138
3	2–5	0.08	72.5	765	780	85
3	3–5	0.06	72.5	785	780	105
Average			72.3	794	771	123

ANALYSIS OF VARIANCE FOR LOAF VOLUME RESPONSE

	Degrees of freedom	Mean squares
Granulations	4	5850.6**
Flours	2	5789.4**
Brans	2	709.8
Granulations × flours	8	552.2
Granulations × brans	8	211.8
Flours × brans	4	450.7
Flours × brans × granulations	16	334.7
Total	44	

** Highly significant.

Water extracts were used to replace a corresponding amount of water in the dough mix. All doughs were then baked; the loaf volumes were as follows:

Extract	Control cc.	Control + extract cc.
Water	630	747
Water	615	730
Water	635	745
Ether	615	637
Alcohol	615	645

This indicates that an ameliorating substance must be present which is soluble in water and at least relatively insoluble in ether and alcohol. This is in agreement with the early work of Willard and Swanson (1911) and White (1913) who found that water extracts of bran increased loaf volume.

Effect of Washed Bran and Cellophane. Washed bran was prepared by tying coarse bran in cheesecloth and washing vigorously under running water for 3 hr. The resulting wet bran was dried as rapidly as

possible at room temperature in a forced-draft air oven. The dried bran was then ground in the Mikropulverizer and separations of different granulations were made on the experimental sifter. This material was then added to white flours and baked in the manner described for unwashed bran. As a control for this work, cellophane ground to corresponding granulations was used. The loaf volumes are given in Tables II and III. Representative photographs are shown in Figures 2 and 3.

TABLE II

EFFECT OF WATER-EXTRACTED BRAN PARTICLE SIZE ON LOAF VOLUME

Flour	Average diameter of bran particles	Loaf volume		
		Washed bran	Control	Response (Diff. + 400)
	mm	*cc*	*cc*	*cc*
1	2.49	468	718	150
2	2.49	608	810	198
3	2.49	440	740	100
Average		505	756	149
1	1.37	485	718	167
2	1.37	635	810	225
3	1.37	460	740	120
Average		527	756	171
1	0.36	550	718	232
2	0.36	713	810	303
3	0.36	613	740	270
Average		625	756	268
1	0.07	595	718	277
2	0.07	735	810	325
3	0.07	590	740	250
Average		640	756	284

ANALYSIS OF VARIANCE FOR LOAF VOLUME RESPONSE

Source of variation	Degrees of freedom	Mean square
Granulations	3	13,845**
Flours	2	6,447**
Interaction	6	475

** Highly significant.

These baking data were treated to statistical analysis by subtracting the control volumes from the corresponding bran or cellophane loaf volumes and adding 400 to this figure. These response values were

TABLE III

EFFECT OF CELLOPHANE PARTICLE SIZE ON LOAF VOLUME

Flour	Av. diameter of cellophane particles	Loaf volume		
		Cellophane	Control	Response (Diff. + 400)
	mm	*cc*	*cc*	*cc*
1	1.02	547	750	197
2	1.02	497	820	77
3	1.02	480	750	130
Average		508	773	135
1	0.67	610	718	292
2	0.67	570	820	150
3	0.67	590	750	240
Average		590	763	227
1	0.27	640	725	315
2	0.27	652	784	268
3	0.27	695	750	345
Average		662	753	309
1	0.06	670	745	325
2	0.06	700	820	280
3	0.06	715	777	338
Average		695	781	314

ANALYSIS OF VARIANCE FOR LOAF VOLUME RESPONSE

Source of variation	Degrees of freedom	Mean squares
Granulations	3	24,848.7**
Flours	2	12,357.3**
Interaction	6	1,098.2
Total	11	

** Highly significant.

analyzed for variance, using the interaction, flours × granulations, as error variance; the results are summarized at the foot of Tables II and III. The correlation coefficient between the figures for washed bran and the average diameter of bran particles was −0.954. Statistical comparison of corresponding cellophane and washed bran loaf volume figures show no significant difference between the two series.

Discussion

Examination of the data on the chemical analyses of the brans used fails to show any factor which might consistently cause the different

granulations of bran to vary in their baking qualities on this basis alone. Examination of the analyses of variance and of the correlation coefficients between bran size and loaf volume shows that the granulation of bran plays an important role in the baking of bread containing bran. Loaf volume of these breads is negatively correlated with bran size, indicating that the size of bran loaves increases as the bran particles are ground finer. This leads to the conclusion, at present realized to some extent in commercial circles, that better whole wheat flours may

Control 1.02 0.67 0.27 0.06

Fig. 2. Loaves of bread baked with various granulations of cellophane. Number under each loaf indicates average diameter in mm.

Control 2.49 1.37 .36 0.07

Fig. 3. Loaves of bread baked with various granulations of washed bran. Number under each loaf indicates average diameter of particle in mm.

be produced by reducing the bran contained therein to as fine a particle size as possible. In view of the possibility of starch granule injury, this may require a grinding of the bran after separation from the endosperm. A striking feature, aside from loaf volume, was the change of color and texture as the bran size became smaller. At the last level of granulation in which bran particles approached flour particle size, it became difficult to distinguish individual bran particles in the finished product, and the grain and texture were similar to those of white bread. This is shown to some extent in Figure 1.

The significant differences between flours indicate, in this series at least, a difference in response of flours to the addition of bran. On the other hand, response to different brans did not differ significantly.

An unusual feature of unwashed bran loaves is their consistently large volume as compared with the washed bran and cellophane loaves. Comparison of the washed bran and cellophane loaves shows that they do not differ significantly, indicating that washed bran has only a "diluting" effect on baking similar to an inert substance such as cellophane. However, even in these two series, the effect of granulation is clearly shown. The presence of two factors in the problem of bran-containing doughs is then indicated. One is detrimental and merely mechanical and is diminished by grinding bran to a finer particle size. The other is an improving factor which is water-soluble, as indicated both by data on washed bran loaves and by work on water extracts of bran. The authors are at present further investigating this factor.

Summary

Three samples of bran were ground so that five different granulations were prepared from each. Granulations varied in average particle size from 0.06 mm in diameter in the finest grinding to 2.66 mm in the coarsest. These brans were superimposed at the rate of 15% on three different bread flours and the resulting mixture baked, using a slightly modified A.A.C.C. procedure.

The effect of the addition of bran on loaf volume varied with the different flours, but the three brans produced similar results.

A high negative correlation was found between loaf volume and bran-particle size. At the level used, coarse bran caused a very significant decrease in loaf volume, while the finest granulations caused a barely significant increase in loaf volume when compared to the corresponding control loaves. Bran contains a water-soluble substance which when added to white flour doughs increases loaf volume. Water-extracted bran decreases loaf volume to about the same extent as cellophane when added to flour at the same stage of granulation. Results indicate that bread of good volume and texture can be produced from flour containing finely ground bran.

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A COMPARATIVE STUDY OF THE DEVELOPMENT OF AMYLASES IN GERMINATING CEREALS [1]

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Most investigations dealing with the development of amylases in germinating cereals have been limited to those cereals adapted to cool climates. For example, the nature of the amylase systems of barley and wheat malts is well understood. In contrast, information relating to the "warm climate" cereals, such as maize and sorghum, is quite limited. The present communication deals with the results found when seven cereals were germinated under comparable conditions. Four of these can be designated as cool-climate cereals—barley, wheat, rye, and oats. Three are representatives of the warm-climate group—maize, sorghum, and rice.

It is well known that the cereals barley and wheat are characterized by high levels of β-amylase when ungerminated and by relatively high levels of both α- and β-amylase when germinated. The researches of Baker and Hulton (1921), Naylor, Spencer, and House (1925), Ohlsson and Uddenberg (1933), and Chrzaszcz and Janicki (1933) demonstrated that rye has amylolytic properties very similar to those of barley and wheat. In contrast, oats, either germinated or not, was found to be deficient in β-amylase (Baker and Hulton, 1929; Ohlsson and Edfeldt, 1933; Chrzaszcz and Janicki, 1933). However, like barley, wheat, and rye, this cereal does have α-amylase activity when germinated.

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With regard to the warm-climate cereals, Patwardhan (1929) found that germinated maize had both starch saccharifying and starch liquefying properties, but that the ratio of saccharifying activity to liquefying activity was much lower than that characteristic of barley malt. Gore and Józsa (1932), and Chrzaszcz and Janicki (1933) found that ungerminated maize had low starch liquefying properties and negligible saccharifying activity. In contrast, Bernstein (1943) advanced the hypothesis that ungerminated maize has β-amylase activity in small degree and a complete absence of α-amylase. In a further report (Bernstein, 1943a), it was shown that germinated maize had both α- and β-amylase activity to an appreciable extent. Sorghum exhibits amylolytic properties similar to those of maize—low starch liquefying and saccharifying properties in the ungerminated state (Patwardhan and Norris, 1928; Gore and Józsa, 1932) and a low ratio of saccharifying to liquefying or to dextrinizing activity when malted (Norris and Viswanath, 1923; Patwardhan and Norris, 1928; Acharya, 1934). Recent studies by Kneen (1944) indicate that the amylase of sorghum is primarily of the α type. The α-amylase was present in ungerminated sorghum in low concentrations and in much greater quantity in the germinated grain.

Many studies have been made of the amylase system of rice, and a few of these were available to the writer. Gore and Józsa (1932) found that ungerminated rice had measurable starch liquefying activity but no saccharifying activity. Karmarkar and Patwardhan (1931) and Venkata Giri and Sreenivasan (1937, 1938) likewise found that ungerminated rice was very low in amylolytic activity. In addition, they showed that a pronounced increase in starch saccharifying and liquefying properties occurred when rice was germinated. This was confirmed by Yamagishi (1938) and by Sreenivasan (1939). The conclusion drawn from the data was that germinated rice had more than one amylase; in fact, Yamagishi (1938) maintained that three independent amylases were present—a starch liquefying, a dextrinizing, and a saccharifying enzyme.

The available data show then that of the seven cereals cited, ungerminated barley, wheat, rye, and oats all have considerable starch saccharifying activity, oats being much lower in this respect than the other three. By comparison, starch saccharifying activity by extracts of ungerminated maize, sorghum, or rice is negligible. All the cereals appear to have some starch liquefying activity (Gore and Józsa, 1932), but of very low degree when compared to that characteristic of their malts. In all cases, germination of these cereals caused a marked increase in starch saccharifying and liquefying (or dextrinizing) activities. Malts of barley, wheat, and rye were found to have all these activities

in high degree, whereas the malts of oats, maize, sorghum, and possibly of rice showed low ratios of saccharifying to liquefying activities.

The general conclusion has been that all germinated cereals have both α- and β-amylase present, but in varying degree and in varying ratio to each other. The present investigation was designed to provide additional data on the nature of the amylase systems of a number of cereals.

Materials and Methods

Grains used for the study were wheat (Cheyenne selection), barley (Wis. ped. 38), maize (Svec Reid), sorghum (Early Kalo), rice (Blue Rose), and unidentified samples of rye and oats. Excepting the rice sample, all were grown under the same environment.

The methods used for germination, drying, sampling, and analysis were essentially those reported by Kneen, Miller, and Sandstedt (1942) —sound grain was selected, steeped to a high moisture level, germinated by the "rag doll" technique, and dried at room temperature with the aid of high speed fans. "Free" and "total" (papain) extractions were made on both the germinated and ungerminated samples. To assist in stabilizing α-amylase (Kneen, Sandstedt, and Hollenbeck, 1943), dilute calcium acetate solution (1 mg/ml) was used instead of water for extractions and for dilutions. Extraction was carried out at 30°C, and when papain was used it was at a 10% level. Extraction times for the various tests are indicated.

The methods employed for evaluating the α- and β-amylase activities of the samples were based on those proposed for barley and wheat by Sandstedt, Kneen, and Blish (1939) and Kneen and Sandstedt (1941). Estimation of the α-amylase activities of ungerminated cereals was made according to the procedure outlined by Kneen, Sandstedt, and Hollenbeck (1943). Calculation of the β-amylase activities of the malts is based on an assumption that the saccharifying potentials of the α-amylases of all the cereals examined are similar to those established for wheat and barley. This has been verified for sorghum (Kneen, 1944).

Results

Amylases of the Ungerminated Grains. Table I shows a comparison of the ungerminated grains. The "free" extracts were 1-hr calcium acetate extracts; the "total" were 18-hr extracts with 10% papain. In conformity with the literature it was found that the saccharifying activities of barley, wheat, and rye were high and that of oats, low. Starch saccharification by the samples of maize, sorghum, and rice was in amounts below the sensitivity of the method. Extracts of all the

TABLE I

THE AMYLOLYTIC ACTIVITY OF UNGERMINATED CEREAL GRAINS

Cereal	Saccharifying activity		β-amylase activity		α-amylase activity	
	"Free"	Total	"Free"	Total	"Free"	Total
	units	units	units	units	units	units
Barley	10.7	29.8	10.7	29.8	0.045	0.058
Wheat	7.5	25.1	7.5	25.1	0.050	0.063
Rye	9.1	17.8	9.1	17.8	0.089	0.111
Oats	0.7	2.4	0.7	2.4	0.262	0.297
Maize	*	trace	*	*	0.101	0.249
Sorghum	*	trace	*	*	0.031	0.127
Rice	*	trace	*	*	0.075	—

* Below the sensitivity of the method used.

samples had α-amylase activity in measurable quantities. The amounts of α-amylase were, however, too low to influence saccharification appreciably, so that for these ungerminated samples "saccharifying units" were synonymous with "β-amylase units."

Approximately one third of the "total" β-amylase of the barley, wheat, and oat samples was readily extractable with dilute calcium acetate solution and about one half of the rye β-amylase.[2] The α-amylase of ungerminated wheat, barley, rye, and oats was somewhat more "soluble" than the β component; *i.e.*, a greater percentage of the papain-extractable enzyme was extracted in 1 hr by the dilute calcium acetate solution. The α-amylase of sorghum and maize was relatively more difficult to extract. No value is given for the total α-amylase of rice. Considerable difficulty was experienced with the papain extracts of this cereal and poor end-points prevented an accurate evaluation of the α-amylase activity.

Amylases of the Germinated Grains. The germination study was conducted using 45-g samples of the grain, a 24-hr steep at room temperature, and germination at 16.5°C for 5 days. In an attempt to obtain a wider spread between the "free" and "total" amylases of these germinated samples, a 15-min calcium acetate extraction was compared with an 18-hr calcium acetate-papain extraction. The data are presented in Table II.

It is apparent from Table II that in all cases germination was coincident with an increase in α-amylase content. In a comparison of the various cereals, however, it is obvious that amylase production is not proportional to sprout length. For example, the germinated oat sample had the longest sprouts but ranked fifth in α-amylase production,

[2] It should be noted that calcium acetate solution, even one as dilute as that used, is a considerably more efficient extractant than distilled water.

TABLE II

THE AMYLOLYTIC ACTIVITY OF GERMINATED CEREALS

Cereal	Sprout length	Saccharifying activity		β-amylase activity		α-amylase activity	
		"Free"	Total	"Free"	Total	"Free"	Total
	mm	units	units	units	units	units	units
Barley	25–30	31.0	39.1	26.5	34.4	90.5	94.0
Wheat	20–30	26.4	34.4	16.5	23.7	197.3	214.7
Rye	20–30	20.1	23.6	15.4	17.6	93.2	119.8
Oats	25–35	2.2	3.0	*	*	53.1	60.3
Maize	15–20	0.24	1.2	*	*	31.1	35.6
Sorghum	10–20	1.8	1.9	*	*	73.4	75.5
Rice	2–5	0.05	0.3	*	0.2	1.4	2.3

* Below the sensitivity of the method used.

showing less than one third the activity of wheat. As would be anticipated, the physiological activities associated with active growth brought about a release of the "bound" β-amylase so that the major portion of the β-amylase in the germinated samples was extracted without the use of papain.

The cereals, maize and sorghum, that showed no measurable β-amylase activity in the ungerminated state likewise showed none when germinated.[3] The β-amylase activity of the germinated rice sample was sufficient to permit evaluation but was only about one hundredth as much as that of wheat, barley, or rye.

A comparison of the data in Tables I and II demonstrates that while there was an increase in the amounts of free β-amylase during the germination of wheat, barley, and rye, the total β-amylase activities of these cereals remained relatively constant. Apparently the oat β-amylase largely disappeared during germination. Since germination resulted in a decrease of total dry weight with all the cereals, the α- and β-amylase data were recalculated on the basis of the original dry weights of the ungerminated samples. This should give an estimate of "absolute" changes in enzyme contents during germination.

"Absolute" Changes in Amylase Content. The recalculated data for the total α- and β-amylase contents of the germinated samples are given in Table III, together with the activities of the ungerminated samples and the dry weight losses resulting from germination. The activity of α-amylase is recorded in terms of α-saccharifying units (saccharifying units minus β-saccharifying units). When this method of calculation is used, it is seen that there was an actual loss of β-amylase during the

[3] Following the completion of the work herein reported, further investigations demonstrated that the germinated samples of oats, maize, and sorghum do have β-amylase activity but of a degree below the sensitivity of the method. An approximate evaluation of the β-amylase contents of three germinated sorghums (Kneen, 1944) showed values of 0.028, 0.093, and 0.24 units of activity. The lowest of these three values is that found for the β-amylase activity of the germinated Early Kalo sample used in the comparative study.

TABLE III

"ABSOLUTE" CHANGES IN THE CONTENTS OF TOTAL β- AND α-AMYLASE DURING GERMINATION OF THE CEREALS

Cereal	Loss of dry weight by germination	Total β-amylase		Total α-amylase	
		Ungerminated	Germinated	Ungerminated	Germinated
	%	units	units	sacc. units	sacc. units
Barley	21.7	29.8	26.9	trace	3.68
Wheat	23.5	25.1	18.1	trace	8.20
Rye	21.7	17.8	13.8	trace	4.69
Oats	26.4	2.4	*	trace	2.22
Maize	18.5	*	*	trace	1.45
Sorghum	19.8	*	*	trace	3.03
Rice	13.8	*	0.2	trace	0.99

* Below the sensitivity of the method used.

germination of those grains originally showing β-amylase activity, this loss being approximately balanced by a corresponding gain in α-saccharifying activity. As with previously recorded data for wheat (Kneen, Miller, and Sandstedt, 1942), there would appear to be some relationship between the two changes. On the other hand, those grains having no measurable β-amylase in the ungerminated state showed normal production of α-amylase during germination and seedling growth.

Comparative Temperature Influence on Germination. The temperature used (16.5°C) is known to be favorable for germination of the cool-climate cereals but probably is far below the optimum for the warm-climate group. Accordingly, the seedling growth and amylase development of wheat and sorghum were compared at four temperatures, 14°, 18°, 24°, and 30°C. Germination of each pair of samples was terminated when the average sprout length of either grain attained approximately 40 mm. Table IV shows the comparative growth rates for the two cereals.

The data of Table IV show clearly the inhibitory influence of low temperature (14°C) on the germination and seedling growth of sorghum. On the other hand, germination was uniformly good at 30°C and the growth rate at this temperature greatly exceeded that of wheat. Growth rates at the intermediate temperatures of 18° and 24°C were approximately equal for the two cereals.

The amylase activities of the various samples were determined and are recorded in Table V. Data were obtained for both free (1-hr) and total (18-hr with 10% papain) extractions. In addition, the activities of the original ungerminated samples are given for comparison.

The amylase activities of those wheat samples germinated at 14°, 18°, and 24°C were very similar. This would be anticipated from the

TABLE IV

RELATIVE GROWTH OF GERMINATING WHEAT AND SORGHUM AT FOUR TEMPERATURES

Days germinated	Approximate average length of sprout							
	14°C		18°C		24°C		30°C	
	Wheat	Sorghum	Wheat	Sorghum	Wheat	Sorghum	Wheat	Sorghum
	mm	mm	mm	mm	mm	mm	mm	mm
2	1	trace	2.5	2	4	4.5	7.5	37.5
3	2	1	9	8	12	15		
4	3.5	2	25	17.5	36	40		
5	5	2	40	40				
6	10	2						
9	45	5						

TABLE V

RELATIVE AMYLASE ACTIVITIES OF WHEAT AND SORGHUM GERMINATED AT FOUR TEMPERATURES

Germination time	Germination temperature	Wheat				Sorghum		
		α-amylase		β-amylase		α-amylase		β-amylase
		"Free"	Total	"Free"	Total	"Free"	Total	"Free" or total
days	°C	units	units	units	units	units	units	units
9	14	250	277	18.2	18.5	31.4	31.0	*
5	18	278	293	18.7	20.4	129	126	*
4	24	250	262	19.4	19.5	109	109	*
2	30	55.3	69.8	14.5	25.9	111	113	*
Ungerminated		0.050	0.063	7.5	25.1	0.031	0.127	*

* Below the sensitivity of the method used.

uniformity of their seedling development. Amylase production as well as growth was rapid at 24°C, but at 30°C the wheat germinated with extreme irregularity and the inhibitory effects of high temperature became evident. It is notable that there was a marked loss of total wheat β-amylase at the three lower germination temperatures; no loss of this component was evident during the restricted growth at 30°C.

The production of α-amylase during the growth of the sorghum samples at the three higher temperatures of 18°, 24°, and 30°C was quite comparable; this, again, is in accord with the relative uniformity of sprout development. Only 2 days at 30°C were required for this development as compared with 5 days at 18°C. The inhibitory influence of the 14°C temperature on sorghum growth was further evidenced by the deficiency in α-amylase development. As in the pre-

vious study, the presence of β-amylase in the samples of germinated sorghum could not be detected by the methods used.

It is apparent that, with one grain, conditions which influence germination and growth have a comparable effect on α-amylase production. However, it is notable that a sprout elongation to approximately 40 mm was accompanied by about twice the amylase production in wheat as in sorghum. Additional data obtained with rice further confirm the observation that with different cereals equal growth rate is not necessarily coincident with equal α-amylase production. A sample of rice was germinated at 30°C for 5 days. The average sprout length at the end of this period approximated 30 mm. After drying, a 1-hr extraction was made and amylase activities were determined. These were found to be 8.9 units of α-amylase and 0.54 units of β-amylase. When compared with any of the other cereals tested (Table II), it is apparent that the growth of germinating rice coincided with relatively minor production of α-amylase. These data likewise provide additional evidence that germinated rice does contain β-amylase, but in relatively small quantity.

Discussion

In a general way the results presented conform to those previously reported in the literature. The chief exception is that the customary methods for the evaluation of β-amylase activity were not sufficiently sensitive to detect the presence of this enzyme in either maize or sorghum. However, a micromethod (Kneen, 1944) did demonstrate the presence of β-amylase. It appears that just as α-amylase is present in cereals, germinated or ungerminated, in amounts varying from traces to considerable quantities, so the presence of β-amylase may be characteristic of all cereals. In some instances the levels of this latter component are so low that they cannot be evaluated by macromethods. The data presented for single samples are not entirely conclusive in themselves but become significant when supplemented with other investigations (Kneen, 1944 and unpublished data).

Other limitations of the comparative germination study warrant emphasis. Wheat appears to be particularly efficient in the production of α-amylase. However, differences in growth temperature optima for the various cereals and the use of single samples do not justify such a conclusion. As a further example while oats are characteristically low in β-amylase, the sample used may have been abnormally low. The results then illustrate the variation which may be expected when the usual methods of amylase evaluation are applied to a miscellaneous group of cereals and to some extent emphasize the limitations of such methods.

Industrially, the findings may have considerable significance if it be assumed that the amylase systems of these germinated cereals can be duplicated by malting procedures. A malt of high potency with regard to both α- and β-amylase could be prepared from either wheat, barley, or rye. Cereals such as oats, maize, sorghum, or rice would be of little value for the production of malts with high saccharifying activity (Lintner value) but certain ones would be adequate for the production of α-amylase. If malts having only the α component were desired, it appears that this could be approximated by malting a selection of sorghum low in β-amylase. Beta-amylase relatively free of the α form can, of course, be obtained from selected samples of the ungerminated cereals. Thus cereal preparations having almost any desired ratio of the two malt amylase components can be made available for use.

Summary

Seven cereals—barley, wheat, rye, oats, maize, sorghum, and rice—were selected, and amylase activities of both the ungerminated and germinated grains determined. Beta-amylase was found in relatively high amounts in ungerminated barley, wheat, and rye and in much less quantity in oats. The method used was not sufficiently sensitive to detect the presence of this enzyme in ungerminated maize, sorghum, or rice. From one third to one half of the total (papain-extractable) amylase was readily extracted by dilute calcium acetate solution. All the ungerminated samples had α-amylase activity but in relatively minor degree.

Germination of the cereals in all cases led to a pronounced increase in α-amylase activity. The β-amylase of barley, wheat, and rye became more readily extractable. The β-amylase activity of germinated rice, though low, could be evaluated, but the levels of this enzyme in germinated oats, maize, and sorghum were below the sensitivity of the customary methods.

The small quantity of β-amylase present in oats largely disappeared during the germination process. When "absolute" changes in amylase activities were calculated for all the samples, it became apparent that with barley, wheat, and rye, a marked loss of β-amylase occurred during germination.

A study of the influence of temperature on the development of amylase activity during the germination of wheat and sorghum was conducted. Temperatures normally considered desirable for malting wheat inhibited sorghum germination. The significance of this relationship in a comparison of amylase development during the germination of different cereals was pointed out.

Industrially, the availability of cereal preparations having almost any desired combination of α- and β-amylase may have some significance. For example, a malt having high α-amylase activity and practical freedom from β-amylase could be prepared from sorghum, and one having both α and β-amylase from barley; the ungerminated barley itself provides a source of β-amylase relatively free from the α form.

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CORRELATION BETWEEN CHEMICAL STRUCTURE OF SOME ORGANIC COMPOUNDS AND THEIR ACTION AS FLOUR IMPROVERS AND AS INHIBITORS OF PROTEOLYTIC ENZYMES [1]

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Jørgensen (1935) has shown that those oxidizing agents which are flour improvers, such as bromates, iodates, and perborates, are also inhibitors of proteolytic enzymes of the papain type, which are now known to be present in sound wheat flour, and he also found that other oxidizing agents, such as chlorates, which are not flour improvers, do not inhibit these proteolytic enzymes. Correlation between flour improvement and inhibition of proteolytic enzymes of the papain type appears to be an established fact. It does not necessarily follow that the action of flour improvers of this type is primarily due to their action as protease inhibitors, although there is much evidence in favor of such a theory (Elion, 1943).

Some authors are more inclined to explain the action of oxidizing and reducing agents on dough by a direct effect on the flour proteins. Elion (1943) described experiments with glutathione, which showed that glutathione had a softening effect on dough made from unheated flour, but that it had no such effect on dough made from heated flour (in which the proteolytic enzymes were inactivated by the heat treatment), while papain had still a softening effect on dough made from this heated flour. These experiments cannot very well be explained if it is assumed that the action of oxidizing and reducing agents is primarily due to a direct effect on the flour proteins.

[1] The terms, "inhibitors of proteolytic enzymes" and "inhibition of proteolytic enzymes," have been used in this paper to avoid a longer, although more correct, description. It should be kept in mind, however, that proteolytic enzymes can be measured only by the effect which they produce. Inhibitors of proteolytic enzymes have the effect of reducing or eliminating proteolytic activity. These inhibitors may act either directly on the enzymes, or they may act indirectly by decomposing any activators of proteolytic enzymes that are present.

While prior to Jørgensen's papers on this subject only certain *oxidizing* agents were known to be flour improvers, Jørgensen found that ascorbic acid, which is a *reducing* agent, also acts both as a flour improver and as an inhibitor of proteolytic enzymes, and he stated that further investigations were required to determine whether ascorbic acid itself possesses these interesting properties, or whether it might actually be the oxidized form of ascorbic acid which is the active compound. Melville and Shattock (1938) found that dehydroascorbic acid is a more efficient flour improver than ascorbic acid and they demonstrated that wheat flour contains an enzyme capable of catalyzing the oxidation of ascorbic acid to dehydroascorbic acid.

The purpose of the present investigation has been to study whether any other organic compounds could be found which also act both as flour improvers and inhibitors of proteolytic enzymes of the papain type, and whether, in the event of positive results, any correlation between the chemical structure of such compounds and their particular action could be recognized.

In his first paper on the inhibition of proteolytic activity, Jørgensen (1935) described only the effectiveness of ascorbic acid, but in his related British patent (1936) he disclosed the action of ascorbic acid and its alkali salts as flour improvers. Noury and Van der Lande (1939), referring apparently to Jørgensen's discovery, gave a somewhat different description of the effective compounds, namely l-ascorbic acid and "its related compounds" or compounds containing "the l-ascorbic acid radical," and in the corresponding French patent (1938) they described the compounds as ascorbic acid and "other enediols." Although Jørgensen himself did not describe compounds other than ascorbic acid and its alkali salts, and Noury and Van der Lande did not present experimental evidence in support of the description "other enediols," the latter authors thus suggested a relation of enediol groups in the molecule to the action of flour improvers. In other fields than that of proteolytic enzymes, relationship of chemical structure to biological action has frequently been described in the literature.

Preliminary experiments led us to presume, contrary to the suggestion of Noury and Van der Lande, that the presence of an enediol group ·C(OH):C(OH). does not alone determine whether a compound will act as a flour improver and further investigations have supported this viewpoint.

Experimental

In our initial experiments the influence of certain compounds on the effect of papain was studied. A simple method to investigate whether a compound inhibits papain has been described by Jørgensen

(1935). In this method gelatin is used as the standard protein material and a papain extract is allowed to react for some time upon a properly buffered gelatin solution at a constant temperature, in the presence or absence of the compound which is to be investigated. Thereafter it is observed whether the gelatin still possesses its property to solidify in a refrigerator.

The papain extract was made by extracting 1 g of papain (Witte) with 100 ml water for 30 min at a temperature of 35°C, whereupon the extract was filtered and the filtrate used. The reaction mixture was made up as follows: 10 ml gelatin solution (made from 20 g gelatin powder to 250 ml water) + 6 ml Sörensen's citrate buffer (pH = 4.95) + 2 ml water (or water and solution of the compound under investigation) + 2 ml papain filtrate. The mixture was kept 1 hour at a temperature of 40°C and then placed in a refrigerator and observed after 15, 30, 45, and 60 min and also the next morning.

The first compound used in these experiments was reductone, which is a strong reducing compound. It proved to be very effective in inhibiting the proteolytic action of papain on gelatin. It was found necessary to use freshly made solutions of reductone, since at the very low concentrations involved, appreciable decomposition occurred in 24 hours.

As stated previously, some doubt arose as to whether the presence of an enediol group in the molecule would be decisive in determining whether a compound will inhibit papain; rather it was presumed that the combination of an enediol group and a carbonyl group next to it: ·C(OH):C(OH)·CO. would be the determining factor. As a matter of fact, this latter group is present both in ascorbic acid and in reductone and an endeavor was made to investigate such compounds that possess an enediol group, but do not have a carbonyl group next to this enediol group. In the event that our presumption were correct, compounds containing an enediol group only should not inhibit papain, whereas compounds having an enediol group next to a carbonyl group should inhibit papain.

Some difficulty was encountered in obtaining compounds having an enediol group only, particularly among the aliphatic compounds. Dimethylacetyleneglycol is such a compound. It is the desmotropic form of acetoin and is believed to be present in small quantity in, and in equilibrium with, acetoin. Experiments with acetoin were entirely negative; the action of papain on gelatin was not inhibited.

The following cyclic compounds were investigated: pyrocatechol, pyrogallol, gallic acid, adrenalin, haematoxylin, carminic acid, and purpurin. While some of these were only partially soluble in water, none of them inhibited papain. All these compounds have one or

more enediol groups in the formula, but no carbonyl group next to the enediol group.

Compounds which have an enediol group adjacent to a carbonyl group were then investigated. Besides reductone, which has already been stated to be a strong inhibitor of papain, the following compounds were tested: dihydroxymaleic acid, reductic acid, and tetrahydroxyquinone. All of these strongly inhibited the proteolytic action of papain, and it was surprising to find that it was immaterial whether the compound was aliphatic or cyclic.

The gelatin method makes a rough quantitative comparison of the effectiveness of different compounds possible, since it permits the use of varying quantities of an inhibitor to determine the minimum amount of each required to inhibit the papain entirely. Several compounds were tested in different concentrations. Under the experimental conditions prevailing, the minimum molar concentrations roughly required to inhibit the papain, so that gelation occurred after one hour in the refrigerator, are given in Table I.

TABLE I

MINIMUM MOLAR CONCENTRATIONS OF DIFFERENT INHIBITORS REQUIRED ENTIRELY TO INHIBIT PAPAIN

Inhibitor	Molar concentration
Potassium bromate	0.0015
Ascorbic acid	0.00015
Reductone	0.00015
Dihydroxymaleic acid	0.0015
Reductic acid	[1]
Tetrahydroxyquinone	[1]

[1] Limits not determined, but less than 0.0015.

In view of these experimental results, it would not be surprising if all compounds containing the group ·C(OH):C(OH)·CO., whether aliphatic or cyclic, would inhibit proteolytic enzymes of the papain type. Some examples of such compounds which were not available at the time of our experiments are oxytetronic acid and rhodizonic acid. Owing to the instability of many of the enediol compounds, no definite series has been used.

It appears to be essential that the group ·C(OH):C(OH)·CO. be present as such. Substitution, such as obtained by replacing a hydrogen atom of the hydroxyl group by sodium, appears to eliminate the compound's property to inhibit papain. Thus, disodiumtetrahydroxyquinone has been found to be inactive, while tetrahydroxyquinone was very active.

In agreement with the proteolytic enzyme theory of Jørgensen

(1935) and of Balls and Hale (1936), compounds having the enediol group next to a carbonyl group were found not only to inhibit papain, but also to act as chemical flour improvers of the bromate and ascorbic acid type. It needs no further emphasis that not all such compounds are permissible in bread, since they may have possible poisonous effects, or have an undesirable influence on other bread properties, such as crumb color (tetrahydroxyquinone).

As an example, baking tests made with reductone will be cited. The flour used was an untreated high protein straight grade Texas flour with 15.0% protein and 0.45% ash. The dough ingredients, based on the flour weight, were yeast 2.2%, salt 1.5%, sugar 3%, shortening 1.5%, water 59%. The fermentation time was 145 min at 30°C (first punch after 75 min, second punch after 45 min and molding after an additional 25 min). The doughs were proofed 55 min at 30° and baked for 35 min at 220°. The addition of 0.0002% of reductone based on the flour weight caused an increase in the bread volume of more than 7%; moreover the bread made with reductone had a better texture, lighter crumb color, and better crust color than the control bread made without reductone.

Further investigations will be required to explain why the presence of the group ·C(OH):C(OH)·CO. in the molecule gives a compound the property of inhibiting proteolytic enzymes of the papain type and acting as chemical flour improvers. Further research will also be required to determine whether the compounds are effective themselves, or whether it is actually some oxidized form which is functioning. Also, the effect of the different compounds described above should be studied with various flours under varying baking conditions. Although it is realized that the present study is not yet conclusive and should be extended in many directions, it is thought that publication at this time may stimulate further research on the mode of action of reducing agents such as glutathione and of flour improvers of the bromate and ascorbic acid type.

Summary

Organic compounds, whether aliphatic or cyclic, which contain the group ·C(OH):C(OH)·CO., were found to act as inhibitors of papain. Examples of such organic compounds, some with strong reducing properties, are given and they were found to act similarly to ascorbic acid. In agreement with the proteolytic enzyme theory of Jørgensen and of Balls and Hale, it has been found that such compounds also act as flour improvers, although their use as flour improvers may be impossible for health—or other reasons.

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AN INSTRUMENT FOR DETERMINING THE COMPRESSIBILITY AND RESISTANCE TO SHEAR OF BAKED PRODUCTS

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(Presented at the Annual Meeting, May 1943; received for publication May 19, 1943)

A number of instruments have been developed to determine the compressibility of bread. Platt (1930) describes a balance type of instrument. L. H. Bailey (1930) describes a simple apparatus in which a sample of bread is compressed in a small box by a standard weight and the compression measured. Platt and Powers (1940) describe the Baker compressibility apparatus in which a stress is applied to the sample by the use of an electric motor and the resulting strain measured. Nikolayev (1941) describes an instrument in which a weight is applied to the surface of a sample and the depression of a semispherical testing finger is measured. All of these instruments, with the exception of the Baker compressibility apparatus, use a fixed weight acting for a fixed time, and to obtain the best results it may be necessary to vary the stress with different samples.

History

An instrument which has been in use in our laboratory for a number of years for determining the compressibility and, also, the resistance to shear of baked products will be described in the present paper. Since

it is almost automatic in operation, a relatively unskilled operator can make tests rapidly and with little error.

This instrument, in its original form, was developed by Bonney, Clifford, and Lepper (1931) to test the softness and ripeness of fruits and vegetables.

Construction and Operation

The construction of the original instrument provided for a vertical shaft, on the bottom of which could be used needles of various sizes or a small rigid plate. Weight was applied to the shaft by delivering mercury at a constant rate into a container on a platform on top of the shaft. The depression of the needle or plate was amplified by an indicator and measured on a scale graduated from 0 to 52. The contact with this indicator was shown by a buzzer and a light operated by flashlight batteries.

Haas [1] (1931) modified the instrument by using a larger rigid plate on the bottom of the shaft and employed it for determining the staling rate of bread. In his work, he used a fixed depression and a varying weight delivered at a constant rate. King, Morris, and Whiteman (1936) used the original instrument for testing the compressibility of cake, employing a varying weight acting for a definite time.

Further modifications have been made which increase the accurateness of the test and the ease of operation (Fig. 1). Mercury was found to be objectionable as a weight, since there was always some spray which prevented using the slices for flavor tests later. The mercury cut-off was operated by hand. Lead shot has been substituted for the mercury, and an electric cut-off added. The flow of shot is adjusted to a standard rate of 500 g per min. Instead of flashlight batteries, 110 volt current is used. The electric cut-off consists of a simple valve held open by a solenoid. A pilot bulb, in series with the solenoid, glows until the contact is made at the lowest limit of the depression. When contact is made, the solenoid current is broken, the pilot light goes out, and the flow of shot is automatically stopped. The compression disk is attached to the shaft by a ball-and-socket joint. This arrangement has been found to give more uniform results, particularly in the case of twisted bread or where molding streaks occur. The ball and socket allows the disk to tilt and tends to average out variations in the firmness of the slices. The disk is 54 mm in diameter and is the largest that could be used on all of the samples tested without covering any of the firmer area close to the crust.

Measuring Compressibility. In testing compressibility, the instrument can be used in three ways: a fixed weight may be allowed to act

[1] L. W. Haas, W. E. Long and Company, Chicago, Ill. Private communication.

for a fixed time and the depression measured; a variable weight may be allowed to act for a fixed time and the depression measured; or the weight required to produce a fixed depression may be determined. The last method has been the most satisfactory. Either hand-sliced or commercially sliced bread can be used. In the case of unsliced bread, slices 25 mm thick are cut in a jig and the crusts trimmed off. The trimmed slice is placed under the disk which is lowered upon it. The indicator is moved to the lower part of the scale and the platform

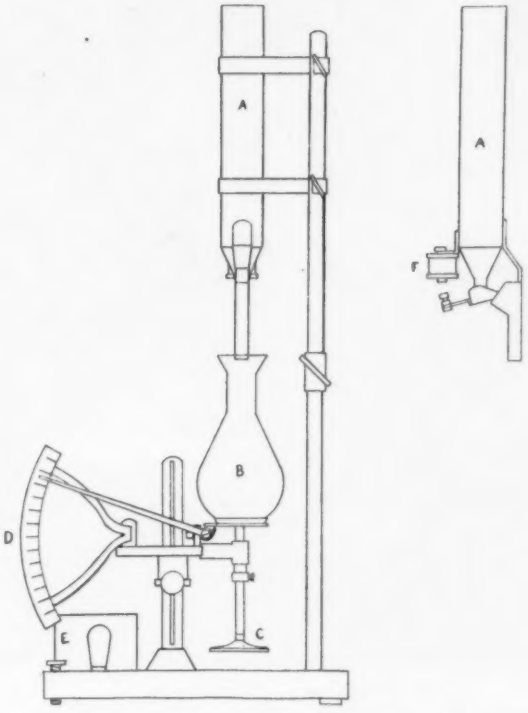

Fig. 1. Diagram of apparatus equipped with compression plate. A—Shot container; B—Receiver; C—Compression disk; D—Scale; E—Relay; F—Solenoid.

adjusted till contact is indicated by the pilot light. The indicator is then set back any desired number of divisions; 25 divisions are satisfactory for slices which are 25 mm thick. This setting corresponds to a depression of the shaft of 4.33 mm. The lever of the shot valve is then raised and the solenoid holds the feed open until contact is again made by depression of the disk. The shot is then weighed. The weight of the disk and shaft assembly is added to that of the shot and container to obtain the total weight effecting the depression. While one slice is being tested, the next may be prepared. Forty mm of each

end of the loaf are discarded. It is preferable to test more than one loaf and average the results. The instrument in use for testing compressibility is shown in Figure 2. Typical figures on the changes in

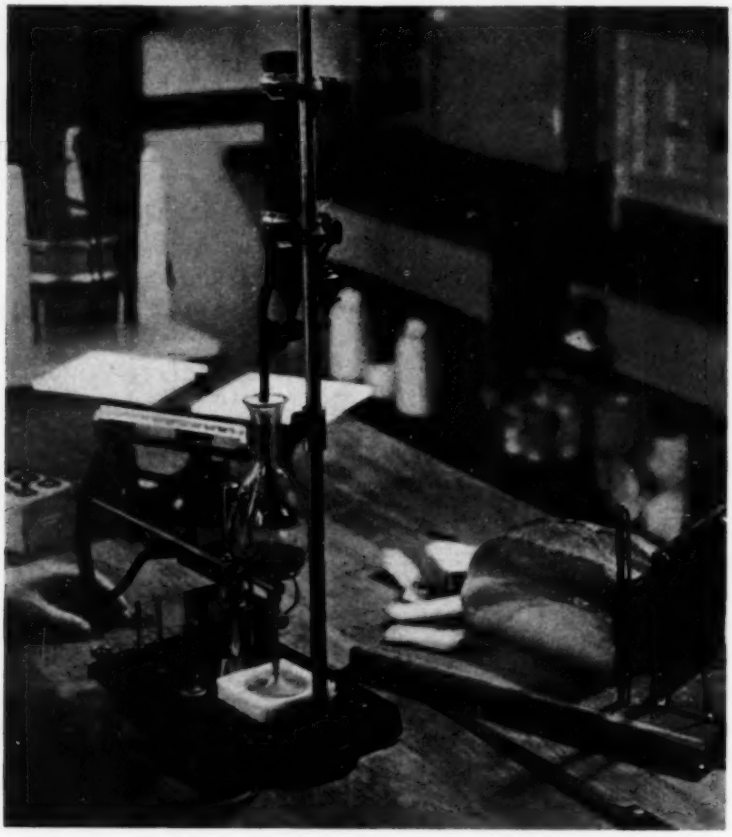

Fig. 2. Instrument in use for testing compressibility.

compressibility of bread during staling are as follows:

Formula No.	Average compressibility per slice at given intervals after baking	
	24 hr	48 hr
	g	g
1	467	626
2	536	703
3	398	529
4	379	532

Testing Shortness of Doughnuts. The same principle of operation has also been used to test the shortness of doughnuts. To obtain uniform results, it has been found necessary to destroy the differences in

cell structure, since some doughnuts are quite close in texture and others are very open. For this purpose, a cylinder with a removable bottom and a telescoping cap is used. The cylinder has a diameter of 25 mm and a depth of 32 mm. The cap has a hole in the top 6.5 mm in diameter. A section of the doughnut weighing 15 g is cut in one piece and inserted in the cylinder, distorting the section as little as possible. Readings are then made through the hole in the top of the cap, using a needle 4.5 mm in diameter.

Testing Resistance to Shear. This apparatus has also been used with another attachment (Fig. 3) for testing the resistance to shear of

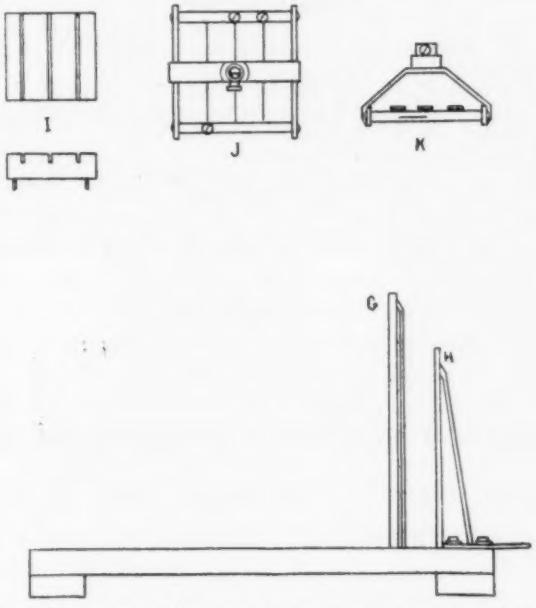

Fig. 3. Shear test equipment and jig for cutting slices. I.—Shear test base; J-K—Shear test head; G—Knife guide; H—Adjustable plate.

bread or other baked products. A frame 3 × 3 inches is rigidly attached to the bottom of the shaft. Across this frame are strung three piano wires, spaced three-fourths inch apart. With the frame is used a platform five-eighths inch high. The top of the platform is one-eighth inch thick and has three slots coinciding in spacing with the wires. These slots extend through the top of the platform and down one-eighth inch into the end supports. With this arrangement, the scale indicator is not used, but only a flexible contact. A slice of bread is placed on the platform and the shot valve opened. When the wires cut through the slice, sufficient drop is obtained to make contact and shut off the feed. Either commercially sliced or hand-sliced bread

can be used. If the bread is hand-sliced, slices 15 mm thick are used. The slices are trimmed exactly 50 mm wide and approximately 60 mm long. The slice is placed on the platform with the wires running across the width. This gives a cut of 150 mm for the three wires.

Summary

An instrument is described which can be used for testing both the compressibility and the resistance to shear of baked products. The method of operation is outlined and typical results obtained in measuring the compressibility of bread are given.

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STUDIES ON THE FRACTIONATION OF ZEIN [1]

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From a study of the solubility behavior of zein with respect to ethyl and amyl alcohol, Donard and Labbé (1902, 1903) grouped zein into three types of proteins: α zein—soluble in amyl and ethyl alcohol; β zein—soluble in ethyl alcohol only; γ zein—insoluble in either alcohol. From a study of the optical rotation of α and β zeins so defined, Lindet and Ammann (1907) concluded that these represent distinct proteins. Watson, Arrhenius, and Williams (1936) obtained, by successive additions of water to a 70% ethanol solution of zein, three distinct and nearly homogeneous fractions. In a later study, Elliott and Williams

[1] Paper No. 2168, Scientific Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis submitted by Robert Theis MacDonald to the faculty of the Graduate School, University of Minnesota, in August, 1941, in partial fulfillment of the requirements for the degree of doctor of philosophy.
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(1939) fractionated corn gluten by successive extractions with methanol and 70% aqueous ethanol.

Zein shows a high solubility in methyl- and ethyl-cellosolve. The present paper deals with the separation of zein into fractions by the successive additions of water to cellosolve solutions of the protein. A study was then made of some of the physicochemical properties of the fractions obtained in this manner.

Since experiment showed that the fractionation of zein by addition of water to solutions of the protein in either methyl or ethyl cellosolve yielded fractions which were entirely comparable to each other, this report will deal only with fractions obtained in the following manner from methyl cellosolve solutions.

Experimental

Preparation and Purification of Zein Fractions. A sample of commercial zein [4] was first purified by an adaptation of the method of Mason and Palmer (1934), wherein the zein was dissolved in 80% ethanol, washed repeatedly with ethylene dichloride or petroleum ether until all color was removed and, after concentrating the solution by removal of part of the alcohol by vacuum distillation, precipitated by dilution in a large volume of 1% sodium chloride solution. The gummy mass so obtained was washed with water to remove part of the salt and the remaining alcohol and was allowed to dry in the air.

When this air-dry zein (moisture content approximately 4%) was suspended in methyl cellosolve, a part of the protein (about 10%) failed to go into solution. This fraction (called Fraction X) was removed by centrifuging and the remaining supernatant solution diluted with solvent to form an approximately 6% (by weight) solution of the protein in methyl cellosolve.

Aliquots of this solution were diluted with various amounts of water and the quantity of protein precipitated was determined. Table I

TABLE I

EXTENT OF PRECIPITATION OF PROTEINS FROM 6% ZEIN SOLUTION IN METHYL CELLOSOLVE BY ADDITION OF WATER IN VARYING AMOUNTS

Percent (by volume) water added	Percent of total protein precipitated
23.0	0
28.5	13.2
33.3	69.6
37.5	91.6
44.4	97.5
50.0	97.7

[4] Commercial zeins were supplied for this work by the American Maize Products Company and by the Corn Products Refining Company.

shows the extent of this precipitation as increasing percentages of water (by volume) were added to the cellosolve solution. It is seen that approximately one third of the soluble protein is precipitated at 31% water content and approximately two thirds is precipitated at 34% water content. These water contents were chosen, then, in order that the total soluble zein should be roughly fractionated into three equal parts. The fraction precipitating at 31% water content was called Fraction A, that removed by increasing the water content to 34% was Fraction B, and that remaining in solution at 34% water content but which could be precipitated by pouring this solution into a large volume of 1% sodium chloride solution was called Fraction C. A number of such fractionations were made and parts of each fraction were repurified by suspending them in water and electrodialyzing nearly free of ash. The protein was collected and air dried, then dissolved in 80% ethanol and precipitated by pouring the concentrated alcohol solution into ethyl ether. The precipitated protein was then dried by washing with anhydrous ethyl ether several times. During this purification process a considerable and varying amount of the original fraction was lost in each case.

It was assumed that during the process of repurification there was no change in such physical properties as micellar weight, optical rotation, peptization, and the like. Table II shows the yields of finally

TABLE II

YIELDS OF REPURIFIED FRACTIONS OF ZEIN FROM ORIGINAL FRACTIONS

Fraction	Weight	
	Before purification	After purification
	g	g
X	37	20
A	60	32
B	54	26
C	58	41
A_1	25	11.5
A_2	17	7.5
A_3	30	27.5

purified samples obtained from original amounts of each fraction so treated. It was on such repurified samples of the various fractions that the subsequent studies were made.

A further fractionation was made on Fraction A obtained as above described. An air-dry sample of this fraction (not repurified) was suspended in methyl cellosolve to form a 6% solution. Approximately 25% failed to go into solution. This was separated and designated Fraction A_1. The remaining solution was brought to 31% (by volume)

of water, the water content at which all of Fraction A had separated in the original fractionation. Only a part of the redissolved Fraction A precipitated at this point. This was collected as Fraction A_2, and the protein left in solution at this water content was collected as Fraction A_3. Each of these three fractions of Fraction A was repurified as already described for the original fractions, and used in the following studies. Amounts of repurified Fractions A_1, A_2, and A_3 obtained from the noted amounts of the original unrepurified fractions are given also in Table II.

The nitrogen and ash contents of these fractions of zein, calculated on a moisture-free basis, are given in Table III.

TABLE III

ASH AND NITROGEN CONTENTS OF THE VARIOUS FRACTIONS OF ZEIN
(Results expressed on moisture-free basis)

Fraction	Ash	Total nitrogen (ash-free basis)
	%	%
X	0.18	15.68
A	0.31	15.68
B	0.15	15.77
C	0.92	15.26
A_1	0.90	12.98
A_2	1.93	14.69
A_3	0.41	15.77

Precipitation Studies. A comparative study was made of the precipitating action of water upon stock solutions of the various zein fractions dissolved in methyl cellosolve (the concentration of protein in these stock solutions is shown in Table IV). In each set of experiments a definite amount of the protein solution was placed in each of a series of test tubes. A definite volume of water was carefully added, equilibrium allowed to be established, and the percent protein precipitated determined by nitrogen analysis of the supernatant solutions. Results are shown in Table IV.

The results shown in section B of Table IV are especially significant. The precipitation curves of Fraction A_2 and A_3 on the one hand, and that of Fraction A_1 on the other hand, point to the existence of two distinct types of protein which, for convenience, will be called Type I (Fractions A_2 and A_3) and Type II (Fraction A_1). The action of added water upon methyl cellosolve solutions of both A_2 and A_3 was very sharp, forming compact coagulums; the addition of water to Fraction A_1 formed a suspension which was stable even upon standing for several hours. Amounts of precipitate recorded refer actually to the amounts of separable coagulum and do not include the material left in suspension as a stable sol.

TABLE IV

FRACTIONAL PRECIPITATION OF VARIOUS ZEIN SAMPLES BY THE ADDITION OF WATER TO THEIR METHYL CELLOSOLVE SOLUTIONS

Fraction	Conc. zein in stock solns.	Percentage of total protein precipitated upon additions of given percentages of water (by volume)					
		28.5	33.3	37.5	41.2	44.4	50.0
	g/100 ml						
Section A							
X	5.4	0	28.7	67.6	82.7	84.5	73.0
A	4.7	0	38.7	78.3	88.6	—	88.8
B	5.5	0	30.0	73.2	78.8	50.9	17.2
C	5.5	0	00.0	47.9	87.0	96.0	99.0
Section B							
A_1	4.8	0	1.0	3.5	3.6	2.0	2.0
A_2	4.7	0	48.1	82.9	93.8	96.1	99.0
A_3	5.7	0	32.6	75.9	89.6	96.0	98.9
Section C							
Mixture, $5A_3 + 1A_1$	6.0	0	7.5	64.7	81.7	82.2	27.5
B_1	4.3	0	50.2	83.1	93.8	91.8	94.9
B_2	3.4	0	0	8.1	4.9	3.5	10.6

If it is assumed that in a mixture of the two types of protein (I and II) in solution, a complex disperse-phase system is formed, then, upon addition of water to such a system, the type of precipitation curve obtained would depend upon the relative quantity of each type present. The following experiment was devised to test the effect of one type upon the other with regard to the character of coagulum formed when their methyl cellosolve solutions were diluted with water.

A mixture of Fractions A_3 and A_1 was dissolved in the ratio of 5 : 1 in methyl cellosolve. Water was added to this stock solution as before. Results are shown in Table IV, section C. In this case, a mixture has been produced which is quite similar to—if not identical with—Fraction B. It should be possible, therefore, to resolve Fraction B into subfractions which would have the precipitation properties characteristic of Type I and Type II.

To effect such a resolution, a 6% solution of Fraction B in methyl cellosolve was prepared and water added up to 40%. The precipitate, called Fraction B_1, which should be of Type I, was separated from the filtrate, which then contained Fraction B_2. Each fraction was air dried and resuspended in methyl cellosolve. The results of precipitation experiments on both of these fractions are given in Table IV, section C, and may be compared with the original Fraction B. It is seen that Fraction B_1 gives a precipitation curve which is quite similar to that of Fraction A_2 or A_3 (Type I) while Fraction B_2 is similar to Fraction A_1 (Type II).

Optical Rotations of Solutions of Fractions of Zein in Ethanol. Lindet and Ammann (1907) found that the α and β zeins, as defined by Donard and Labbé (1903), showed varying specific optical rotations when dissolved in ethanol solutions of varying water content. Also, the rotations of the two fractions were not identical when dissolved in the same ethanol-water mixture. From this they concluded that these zeins represented distinct proteins. Table V gives the $[\alpha]_D^{23}$ values for

TABLE V

SPECIFIC OPTICAL ROTATIONS OF ZEIN FRACTIONS A_3, B, C, AND A_1 AT 23°C IN DIFFERENT CONCENTRATIONS OF ETHANOL

(Values for α and β zein by Lindet and Ammann (1907) for comparison)

Ethanol concentration	Specific optical rotation $[\alpha]_D^{23}$					
	Lindet and Ammann		Zein fractions			
	α zein	β zein	A_3	B	C	A_1
%	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*
70	29.6	40.0	39.4	36.0	32.5	23.9
80	29.6	40.0	35.8	—	32.5	23.9
90	23.1	22.1	23.3	25.2	21.7	18.3

four of our fractions together with those of Lindet and Ammann for their α and β zeins. Fractions C and A_3 give values which agree fairly well with those for the α and β fractions, respectively, of Lindet and Ammann. Fraction A_1, however, must be considered as distinctly different from their fractions. Lipatov and Putilova (1936), in their study of gelatin fractions, found the less soluble (larger molecular) fractions showed correspondingly higher specific rotations. We should expect Fraction A_3 to have a higher molecular weight than Fraction A_1 on this basis. Osmotic pressure measurements showed this to be the case.

Osmotic Pressure Studies. Osmotic pressures of Fractions A_3, B, C, and A_1 dissolved in methyl cellosolve were determined at three concentrations. The instrument used was similar to that described by Dobry (1935). As collodion is soluble in methyl cellosolve, it was necessary to use a denitrated collodion membrane. Denitration was accomplished by the use of ammonium hydrogen sulfide according to the method described by Jilk (1937). Experimental data are shown in Table VI and Figure 1.

The relationships observed between osmotic pressure and concentration for the various fractions of zein conform with the expression

$$P = AC + BC^2,$$

TABLE VI

OSMOTIC PRESSURE DATA OBTAINED WITH ZEIN FRACTIONS B, C, A_1, AND A_3 IN METHYL CELLOSOLVE SOLUTION

Protein concentration g/liter soln	Osmotic pressure [1] cm water (25°C)	Osmotic pressure/conc.
	FRACTION C	
19.15	14.10	0.78
9.58	7.73	0.81
4.79	4.04	0.84
	FRACTION A_3	
19.36	18.02	0.93
9.68	7.40	0.76
4.84	3.13	0.65
	FRACTION A_1	
9.43	6.28	0.67
4.72	4.16	0.88
2.36	2.31	0.98
	FRACTION B	
19.25	18.69	0.97
9.63	7.49	0.78
4.81	3.39	0.71

[1] Obtained by multiplying the difference in hydrostatic levels between solution and solvent by the density of solution.

where P is the osmotic pressure, C the concentration, and B and A are constants which represent, respectively, the slope and intercepts of straight lines obtained when P/C is plotted against C. Although the micellar weights are calculated from values of the intercepts, the fractions also appear to be characteristically differentiated by the slopes so obtained. Thus Fractions A_3 and A_1 which show marked differences with respect to specific optical rotation and precipitation behavior are likewise sharply marked in their osmotic behavior by a reversal in sign of slope. The method of least squares was used to determine the most probable value of the intercept. The intercept represents the limiting value of $(P/C)_{C \to 0}$. The molecular weight is then given by the expression

$$M = \frac{22.41 \times (1 + .00367\, t)}{9.651 \times 10^{-4} \times \text{intercept}}$$

where t is expressed in degrees centigrade, P is expressed in centimeters of water, and C is expressed in grams of solute per 1000 ml of solution. Table VII gives the intercepts found and the corresponding calculated molecular weights.

Discussion of Results

In the precipitation study it was found that Fraction B consists of a mixture of Type I and Type II proteins. Upon calculating the

molecular weight for a mixture composed of 85% of component of molecular weight 45,000 (as for Fraction A_3) and 15% of component of molecular weight 23,330 (as for Fraction A_1), a value of 41,750 is obtained. This corresponds closely to the observed value of 41,650

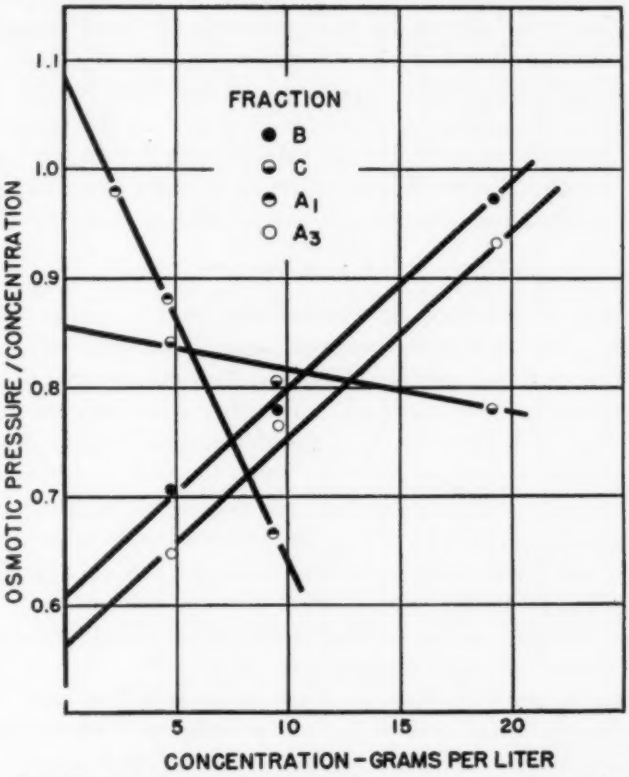

Fig. 1. Osmotic pressure-concentration relationships for various fractionated and refractionated protein products obtained from methyl cellosolve solutions of zein. Fractions B and C are originally obtained fractions, whereas Fractions A_1 and A_2 are refractionated products obtained from Fraction A.

actually found for Fraction B. It may be concluded that Fraction B consists of Type I and Type II proteins in the ratio of 85 to 15.

One of the surprising results of the osmotic pressure studies is the large difference in molecular weight between zein Fractions B and C

TABLE VII

MOLECULAR WEIGHTS OF ZEIN FRACTIONS A_3, B, C, AND A_1

Fraction	Intercept	Molecular weight
A_3	0.563	45,030
B	0.609	41,650
C	0.855	29,650
A_1	1.087	23,330

(Fig. 1). This indicates that the separation of these two fractions has been rather sharp and distinct. The osmotic pressure studies have further corroborated the results obtained with optical rotation and peptization studies. By assuming no change in physical properties during the repurification process and that zein Fractions C, A_2, and A_3 are free of any material similar to Fraction A_1 (Type II protein); also, that 15% of Fraction B, 10% of Fraction X, and 5% of Fraction A (rough approximations based upon peptization data) consist of a component such as Fraction A_1, it can be estimated on the basis of yields of air-dry fractions that 65% of the total protein in zein is a fraction of molecular weight 45,000, 25% is a fraction of molecular weight 30,000, and 10% is a fraction of molecular weight 23,350.

Summary

A method is described of fractionating zein in methyl cellosolve solution by addition of water wherein several fractions have been obtained. One of these fractions (Fraction A) was subjected to a process of refractionation which resulted in a further set of subfractions.

Precipitation studies on the various fractions indicated that two types of protein are present in zein: Type I, which is hydrophobic, is precipitated as a compact coagulum, whereas Type II, which is hydrophilic, is not precipitable by water but forms a stable suspension when thrown out of clear solution. These two types of zein protein were found to differ markedly in their total nitrogen content.

Optical rotation studies on four of the zein fractions pointed to the existence of at least three components.

Osmotic pressure studies on these same zein fractions indicated considerable variation in their molecular weights. These results, together with information obtained from peptization studies, and from the weights of individual fractions obtained in the original separation, lead to the conclusion that zein contains components which fall roughly into three groups: (1) 65% of the total protein consists of a fraction of molecular weight 45,000 (this is slightly higher than the value of 38,000 reported by Elliott and Williams, 1939); (2) 25% of the total protein is a fraction of molecular weight of 30,000; (3) 10% of the total protein is a fraction of molecular weight 23,350. The third protein fraction is of Type II whereas the others are of Type I.

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BOOK REVIEWS

Cellulose and Cellulose Derivatives. Volume V. High Polymers.
Emil Ott. xx and 1176 pp. Interscience Publishers, Inc., New York, N. Y. 1943. Price $15.

This volume is a monograph written by 35 specialists located in the United States. In the introduction, the editor states: "The object of this book is to have in a unified presentation the most important modern scientific and technical information concerning cellulose and its derivatives and to have this information in such form that it becomes a thorough introduction for work on any cellulose problem by any person with reasonably wide general technical training." The historical approach has been almost entirely neglected and only sufficient technical detail has been included as was considered essential for a broad and reasonably thorough understanding of the whole field.

The contents are arranged into 10 chapters, each with many subdivisions. The chapter outline which follows shows the logical order of treatment of the various topics and indicates the scope of the volume.

Chapter I. Occurrence of Cellulose—significance of terms, formation of cellulose, identification of cellulose, industrial uses of cellulose, sources of cellulose, natural occurrence of combined cellulose.

Chapter II. Chemical Nature of Cellulose and Its Derivatives—historical survey, chain structure, end groups, chemical significance of polymolecularity, nature of the association between carbohydrate and lignin in wood, influence of hemicelluloses in pulps, base-exchange properties, cellulose tests, degradation of cellulose.

Chapter III. Structure and Properties of Cellulose Fibers—X-ray examination, structure of wood, microscopic investigation, mercerization, the distribution of submicroscopic metal crystals in fibers, beating and hydration of paper fibers, sorption of water and other vapors by cellulose.

Chapter IV. Carbohydrates Normally Associated with Cellulose in Nature—sugars found in hemicelluloses and related polysaccharides, hemicelluloses, pectin.

Chapter V. Lignin and Other Noncarbohydrates—occurrence, determination, and physical properties of lignin; isolation of lignin; structure of lignin; nature of the other noncarbohydrate compounds in wood and straw.

Chapter VI. Preparation of Cellulose from Its Natural Sources—wood pulp, cotton linters, rags, bast fibers, straw, grasses, and similar materials.

Chapter VII. Bleaching and Purification of Cellulose—bleaching of cellulose textiles and rags, bleaching of acid-cooked wood pulp, multi-stage bleaching, chlorination followed by hypochlorite bleaching, bleaching of alkaline-cooked pulps, washing of pulp, miscellaneous bleaching agents and processes, bleaching and purification of pulp for special purposes, literature reviews and acknowledgment.

Chapter VIII. Derivatives of Cellulose—kinetics and equilibria involved in cellulose reactions, inorganic esters, cellulose organic acid esters, alkali and other metal derivatives, cellulose ethers, xanthates.

Chapter IX. Physical Properties of Cellulose and Its Derivatives—solubility, thermodynamic properties of solutions of long-chain compounds, determination of molecular weight, influence of polymolecularity on physical properties, theory of the viscosity of dilute solutions of long-chain compounds, practical applications of viscosity, elasticity, and strength.

Chapter X. Technical Applications of the Physical Properties of Cellulose and Its Derivatives—natural cellulose, regenerated cellulose and rayon, cellulose derivatives, future applications.

Many of the contributors are engaged in industry or working in research institutions supported by industrial organizations so that the presentation of industrial processes is up-to-date. Recent references which provide an adequate description of the topic under consideration have been given preference over earlier references. Author and subject indices are included.

This volume sets a high standard, and the contributors, editor, and publishers are to be congratulated in providing a modern presentation of the chemistry and technology of cellulose which will be an invaluable reference work for several years to come.

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The Constituents of Wheat and Wheat Products. By C. H. Bailey. 332 pp. Reinhold Publishing Corporation, New York. 1944. Price $6.50.

In his introduction the author says that this book is intended to cover "what might be termed the descriptive biochemistry of wheat and wheat products," and that it is merely "a report of progress." The reader will soon discover that the book is, essentially, a complete historical review, presented objectively and impartially, and including nearly every contribution to our knowledge of wheat constituents that has been reported in the literature up to 1943, starting with Becarri's famous "gluten washing" experiment which was announced in 1728 and published in 1745.

As anticipated by the author, in the introduction, some readers will consider that there is too much detail in the recording of numerous items which are of historical interest only, and are obviously of no practical value in the light of current knowledge. In this reviewer's opinion, however, this is a minor matter. Of far greater importance is the painstaking thoroughness with which the entire world literature has been covered. All cereal technologists will gratefully acknowledge the real service that Dr. Bailey has rendered in making such a comprehensive and unique compilation conveniently available to them.

There are 16 chapters, covering the known and reported constituents of wheat. Seven chapters deal with the nitrogen compounds, and three with the carbohydrates. The remaining six are concerned, respectively, with the lipids, minerals, halogens (together with sulfur and selenium), acidity, pigments, and vitamins.

Some readers will question the propriety of referring to the use of the Kjeldahl method as "Kjeldahling." If this becomes standard practice we may expect eventually to see statements to the effect that a substance was "Sorensened," "Fischered," or "Van Slyked."

Literature citations are complete. There is an author index, and the subject index is adequate. A number of typographical errors are noted, but this is quite excusable under existing circumstances.

Every cereal chemist will wish to own a copy of this excellent book, which is the first and only one of its type in existence. The introduction contains a hint that Dr. Bailey is planning another volume, which will deal with the "dynamic biochemistry" of wheat and will consider enzymes, bread making, wheat processing, etc. Such a volume would be enthusiastically welcomed, and it is hoped that it will eventually make its appearance.

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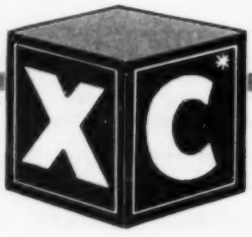

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"Whad'ya Know, Brother, About BULK?"

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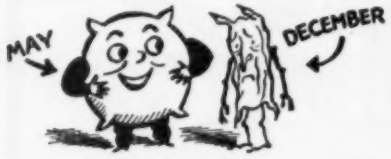

Take the bulkiness of salt—specific gravity—which may vary rather widely. A survey of various salt products from different sections has shown that even salt of the same grade can vary up to 25% in bulkiness over a period of months.

Diamond Crystal processing engineers have eliminated this variation to a considerable extent through rigid control of crystallizing conditions and the most modern screening methods. Thus the various grades of Diamond Crystal Salt will not vary more than 4% plus or minus —usually not over 2%.

If you, as a food processor, are applying salt by volume—through a hopper, for example, as in salting soda crackers, potato chips, and similar products—you can only achieve really accurate flavor control by using a salt that varies but slightly in bulkiness. Why not check the density of each shipment of salt you are using and see how much it varies in bulk?

NEED HELP? HERE IT IS!

If this is the solution to that difficult variable-flavor problem, write our Director of Technical Service. He will be glad to help you find a cure. Diamond Crystal, Dept. M-4, St. Clair, Michigan.

DIAMOND CRYSTAL *ALBERGER PROCESS* **SALT**

You CAN DEPEND UPON THESE TOP-NOTCH SHORTENINGS . . . . .

PRIMEX B&C

An all-hydrogenated vegetable oil shortening of exceptional stability. Excellent for all deep frying purposes. Preferred by biscuit and cracker bakers, manufacturers of prepared biscuit, pie crust, and doughnut flours, and makers of other food products where rancidity troubles are to be avoided.

SWEETEX

The "High-Ratio" shortening. Especially designed to permit bakers to produce "High-Ratio" cakes, icings, and sweet yeast goods with superior eating and keeping qualities.

PRIMEX

The all-hydrogenated shortening "that sets the standard." A quality shortening especially recommended for doughnut frying, for pies, cookies and bread, and for other shortening purposes.

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DOUGH ROLLED 3/8"
DOUGH ROLLED 1/4"
DOUGH ROLLED 3/8"

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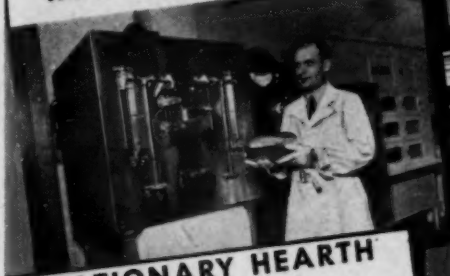

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